Ion mobility spectrometry-mass spectrometry (IMS-MS) of small molecules: separating and assigning structures to ions

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(ABSTRACT)

The phenomenon of ion mobility (IM), the movement/transport of charged particles under the influence of an electric field, was first observed in the early twentieth Century and harnessed later in ion mobility spectrometry (IMS). There have been rapid advances in instrumental design, experimental methods and theory together with contributions from computational chemistry and gas-phase ion chemistry which have diversified the range of potential applications of contemporary IMS techniques. Whilst IMS-mass spectrometry (IMS-MS) has recently been recognized for having significant research/applied industrial potential and encompasses multi-/cross-disciplinary areas of science, the applications and impact from decades of research are only now beginning to be utilised for 'small molecule' species. This review focuses on the application of IMS-MS to 'small molecule' species typically used in drug discovery (from 100 to 500 Da) including an assessment of the limitations and possibilities of the technique. Potential future developments in instrumental design, experimental methods and applications are addressed.

The typical application of IMS-MS in relation to small molecules has been to separate species in fairly uniform molecular classes such as mixture analysis, including metabolites. Separation of similar species has historically been challenging using IMS as the resolving power, R, has been low (from 3-100) and the differences in collision cross-sections that could be measured have been relatively small, so instrument and method development has often focused on increasing resolving power. However, IMS-MS has a range of other potential applications that are examined in this review where it displays unique advantages, including: determination of small molecule structure from drift time, 'small molecule' separation in achiral and chiral mixtures, improvement in selectivity, identification of carbohydrate isomers, metabonomics, and for understanding the size and shape of small molecules. This review provides a broad but selective overview of current literature, concentrating on IMS-MS, not solely IMS, and small molecule applications.

Keywords: ion-mobility mass spectrometry; ion mobility spectrometry; mass spectrometry; small molecule; mass-mobility correlation; collision cross-section; ion mobility; FAIMS; drift-time; travelling wave; structural; computational; differential mobility spectrometry; differential mobility analyzer

I. INTRODUCTION TO IMS

The existence of ions in the gas-phase was first discovered when investigating changes in the electrical conductance of air (Thomson, 1903) which had previously been thought to be an electrical insulator. Further work established the generation of ions by UV and X-ray from work by Thomson and Rutherford (1896), Roentgen (1896) and Rutherford (1897)). The rudimentary scientific tools available at the time did not allow a comprehensive understanding of ion behaviour but the behaviour of simple gas-phase ions in a weak electric field was further elucidated by Langevin (1905), who demonstrated

that air was a mixture of gases and developed models that described these simpler systems remarkably well.

Thomson and Aston later developed the first mass spectrometer (Aston, 1919) and further research focussed on low pressure studies of ion-molecule systems that, although not directly involving typical ion mobility pressure regimes, accrued knowledge in the behaviour of ion motion in a partial vacuum.

The rapid uptake of IMS in military and forensic applications (Zolotov, 2006) benefited from the relatively high proton affinity of the analytes (chemical warfare agents, explosives and illicit drugs), low detection limits and miniaturisation of IMS instrumentation. IMS instrumentation was re-designed for the field, used internal calibrant standards and simplified user interfaces allowed their use by non-scientist military and security personnel. IMS instrumentation is now ubiquitous in handheld forensics, cleaning validation and military applications (from border control to explosive testing in war zones).

Separation in IMS occurs rapidly, in milliseconds, rather than seconds as in chromatography, so IMS is now beginning to be recognised as a powerful separation step which can be utilised post-ionisation using a range of equipment, benefits from robust day-to-day operation and allows size and shape separation and measurement of analytes that cannot easily be derived using other techniques. Miniaturisation, demonstrated so far primarily in overtone IMS and microfabricated Field Asymmetric IMS (FAIMS), is a highly attractive feature and IMS typically has a low detection limit of nanograms and does not require expensive and environmentally damaging solvents.

II. INTRODUCTION TO IMS-MS

The most important aspect of the combination of an IMS separation (typically occurring in the millisecond time-frame) and MS detection (typically occuring in the microsecond time-frame) is that it allows an additional separation step to be obtained on a MS time-frame (e.g. in addition to liquid chromatography), without compromising the speed of MS detection.

The work of McDaniel and Martin (1960), Kebarle (1965) and Hogg (1965), Albritton et al., (1968) and later Crompton and Milloy (1977) developed the recognizable configuration of linear drift-tube IMS (DT-IMS). The combination of ion mobility and mass spectrometry allowed more complex studies to be conducted in order to develop models of ion mobility.

IMS was first hyphenated to a mass spectrometer by Barnes, McDaniel and Martin (1961) using a magnetic sector mass spectrometer; subsequently McAffee and Edelson (1963) described hyphenation to a time-of-flight (TOF) MS. The coupling of IMS with a TOF mass spectrometer is particularly appropriate because the TOF mass spectrometer has the fastest data acquisition rate of any mass spectrometer and can acquire many spectra on the microsecond scale whilst the IMS analytes arrive on a millisecond timescale.

A review by Hill et al. (2007) describes the early development of IMS-MS with significant contributions and discoveries including those from Bowers, Kemper, Clemmer and Kebarle. IMS has since been interfaced to quadrupole mass spectrometers (Karasek et al., 1976; Wu et al., 1998), quadrupole ion traps (Creaser et al., 2000), linear ion traps and Fourier-transform ion cyclotron mass spectrometers (Bluhm et al., 2000) which is beneficial in terms of a wider linear dynamic range and increased mass spectral resolution. Clemmer et al., (1997) and Creaser et al., (2000) also reversed the stages to produce a MS-IMS design that traps and stores ions in an initial quadrupole ion trap for subsequent ion mobility measurements which has the advantage of pre-concentrating low abundant components.

Recently IMS has been hyphenated to mass spectrometry systems and the availability of commercially supported instrumentation has arguably led to a rapid increase in the number of publications on IMS-MS, as shown in FIGURE 1. FAIMS, a type of differential mobility spectrometry, originated in Russia in the early 1980s (Gorshkov, 1982) emerged as an analytical tool (Buryakov et al., 1993), and was later commercialised by Ionalytics (Selectra, 2003), as a front-end accessory for MDS Sciex (Concord, Ontario, Canada) mass spectrometer systems (2004), Thermo FAIMS, (2007) and Owlstone Nanotechnologies FAIMS. Waters Inc. (Milford, MA) launched the first generation Synapt Triwave travelling wave IMS system in 2006 and updated with a second generation instrument with improved resolution (G2, 2009) and improvements to sensitivity (G2S, 2011). AB Sciex launched the

SelexION ion mobility device in 2011 for their triple quadrupole mass spectrometer and quadrupole-trap mass spectrometer.

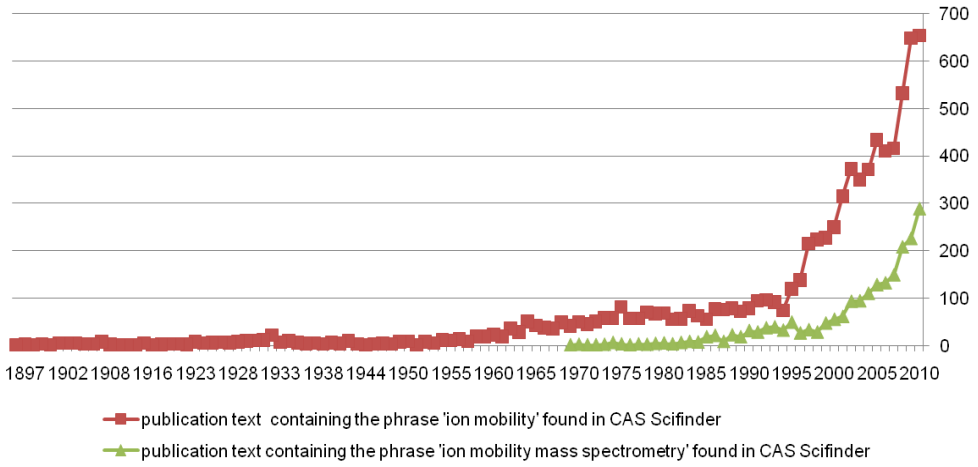

***FIGURE* 1.** Chart showing number of publications containing the phrases "ion mobility" and "ion mobility mass spectrometry" obtained using CAS Scifinder.

There has been limited recognition of the potential of IMS-MS analysis for 'rule-of-5' type small-molecules (Lipinski et al., 1997) outside of traditional IMS analyte classes (e.g. explosives, chemical warfare and illicit drugs). The historical lack of interest and sporadic periods of development in IMS-MS for small molecule applications seems most likely to be due to perceived poor resolution, strengths in competing chromatographic techniques and weaknesses in the robustness of IMS regarding a poor linear range (Turner and Brokenshire, 1994), "memory effects" from contamination (Gehrke, 2001) and interference from matrices.

In contrast IMS-MS has been applied to biomolecules including peptides (Harvey, Macphee & Barran , 2011), proteins (60 kDa-150 kDa) and large protein complexes (1- 4 MDa) (Uetrecht et al., 2010) particularly after the application of non-covalent mass spectrometry conditions to IMS-MS. Using these especially gentle electrospray ionisation conditions is believed to maintain the weak (cooperative) molecular interactions present in many biomolecular structures and thus avoid fragmentation. Whilst IMS-MS typically lacks the resolution (1% error or 10 nm^2) of x-ray or NMR for large molecular weight biomolecules it enables analysis using smaller amount of material, allows analysis of structures >100 kDa that are difficult to analyse by NMR and is, arguably, as realistic an environment as x-ray structures due to lattice effects present. IMS-MS has, therefore, been utilised as a tool to probe the stabilisation of proteins in the presence of ligands and metals (as a unique method of metal speciation; Souza Pessôa et al., 2011), protein-protein-interactions, protein mutants and their structural consequences as well as protein unfolding via various means of ion activation (Jurneczko and Barran, 2011). In addition to detailed understanding of individual biomolecular systems, IMS-MS has also been applied to more high-throughput analytical approaches including screening of phosphorylated peptides (Thalassinos et al., 2009), identification and separation of chemically cross-linked peptides (Santos et al., 2010) and combining topology in protein substructures with proteomics data (Zhou and Robinson, 2010). This review does not attempt to summarise biomolecule analysis by IMS-MS, which is covered by many authors, but rather to contrast the adoption of IMS-MS in biomolecule analysis and focuses on small molecule applications.

III. DIFFERENCES IN PERFORMANCE OF IMS-MS AND IMS

A. The ion efficiency and resolution challenge

Some of the key differences between IMS and hyphenated IMS-MS include the pressure regime in the ion mobility cell, the size of the instrument and the typical ionisation source. Whilst specifications and performance in IMS may be indicative of those in IMS-MS there are some technical reasons why this may not follow. It is critical to note that the results may well be different in IMS-MS compared to IMS especially due to sensitivity issues and pressure regime changes from IMS to MS stages.

In IMS-MS there are often two main challenges (i) to utilise all the ions from the ionisation source, (especially in a pulsed IMS separation such as drift-time IMS, but not a challenge in DMS or FAIMS which typically have a 100% duty cycle if a single transmission voltage is selected) and (ii) elimination of all neutral species whilst ensuring transmission of ions to the MS stages to maintain sensitivity. Traditionally DT-IMS-MS sensitivity has been estimated to be inversely proportional to the IMS resolving power squared; ion losses at the IMS exit aperture ranging from 99 to 99.9% (Tang et al., 2005) and ion introduction losses being between 99.6 to 99.9% (Belov et al., 2008). The desire to increase the gas pressure in the DT-IMS cell to increase resolution must therefore be balanced with the possibility that it may well reduce ion transmission by requiring a reduction of the aperture size in the interface from IMS to MS. The different pressure regimes in IMS systems are described in Table 1.

For DT-IMS-MS the drift gas pressure must be increased proportionally to electric field strength in order to maintain a low E/N ratio (<2 x 10^{-17} Vcm^2), required to obtain field-independent mobilities for which the simplified Mason-Schamp equation (Mason and McDaniel, 1988) holds:

$$K = (3q/16N)(2\pi/\mu kT)^{1/2} 1/\Omega \qquad (1)$$

Where *K* is the ion mobility, q is the ionic charge, N is the buffer gas density, μ is the reduced mass of the buffer gas and the ion, k is the Boltzman constant, T is the temperature and Ω is the collision cross-section (CCS).

For Travelling Wave Ion Mobility-MS (TWIMS-MS) the gas pressure must also be increased proportionally to the electric field, such that:

$$K = K_o N_o / N = K P_o T / (P T_o) \qquad (2)$$

where K is the ion mobility, N is the buffer gas density, N_o is the Loschmidt number (the value of *N* at standard temperature (T_o = 273K) and pressure (P = 1 Atm) and K_o is the reduced ion mobility.

TABLE 1. Pressure regimes in typical IMS systems.

Type of IMS system	Pressure regime	Typical operating pressure
Ambient DT-IMS	Ambient pressure	1000 mbar (Kanu et al., 2008)
Reduced pressure DT-IMS	Reduced pressure	10^{-5} (Ruotolo et al., 2002b) to 1.3 (Valentine et al., 2001) mbar
FAIMS or DMS	Ambient pressure->ambient pressure	400 to 1571 mbar (Kolakowski and Mester, 2007)
Travelling wave IMS	Reduced pressure	0.5 mbar (Waters Synapt G1) to >3 mbar (Waters Synapt G2) (Giles et al., 2011)
Differential mobility analysis	Ambient pressure	1013 mbar

Approaches to maximise sensitivity include utilising quadrupole and octopole ion traps (Henderson et al., 1999;Creaser et al., 2000; Myung et al., 2003) and electrodynamic ion funnels (Wyttenbach et al., 2001) to accumulate and introduce ions efficiently from the ion source to increase the sensitivity of DT-IMS. Multiplexing approaches including Hadamard (Clowers et al., 2006) and Fourier-type (Tarver, 2004) gating techniques have also been utilised for increasing the sensitivity of DTIMS, by increasing the frequency of ion injection events and thus increasing the quantity of ions injected into DT-IMS by up to 50%. Most of these approaches have been integrated into full IMS-MS systems with dramatic improvements in sensitivity.

There is some uncertainty in the identification of the ions in their transmission through the IM cell and it seems clear that hyphenating IMS and MS allows a more comprehensive understanding of the

ionisation processes and fragmentation pathways in IMS. For example, when using a radioactive ^{63}Ni cell, proton transfer to the analytes should lead to protonated monomer and dimer ions; however, without a mass spectrometer as the detector, the identification of the ions in the ion cell cannot be unambiguously ascertained. Indeed, a comparison of limonene and 2-nonanone by IMS and IMS-MS resulted in a variety of unexpected fragments and ions, resulting in a non-trivial IMS spectrum and making interpretation of the results difficult (Vautz et al., 2010). Any unambiguous identification of ions in IMS will be important both in structural measurements (size, shape and topology) and for comparison of related IMS techniques (e.g. TWIMS-MS) that often use historical DT-IMS-MS data as calibration standards in ion mobility and collision cross-section calculations.

B. Features of IMS techniques utilised in IMS-MS

IMS-MS potentially provides a number of advantages over and above IMS including:

i) as a rapid gas-phase separation step before mass spectrometryanalysis,
ii) the identification of ions subsequent to characteristic drift times by comparison with data acquired under comparable conditions,
iii) measurements of collision cross-sections and derivation of further information about size and shape, either by comparison with computational modeling or by analysis within a series of compounds
iv) better characterisation of ion and ion-neutral ion mobilities by simultaneous acquisition of mass spectrometry data, and
v) better characterisation of ionisation (Tang et al., 2006) and fragmentation pathways via a better understanding of gas-phase ion structures

IMS has been hyphenated to liquid chromatography, gas chromatography (Baim and Hill, 1982; Snyder et al., 1993), super-critical fluid chromatography (Eatherton et al., 1986; Huang et al., 1991), ions produced via matrix assisted laser desorption ionisation (Jackson et al., 2007), desorption electrospray ionisation (Weston et al., 2005), pulsed corona discharge ionisation (Hill and Thomas, 2003) and miniaturised to microchip scale (Shvartsburg et al., 2009b).

IMS-MS systems are typically composed of four stages (Figure 2):

1) an ion source e.g. MALDI or electrospray that generates ions. Electrospray ion sources have been preferred for retaining native-like structures in biological systems.
2) an IMS cell, where charged particles migrate under the influence of an electric field.
3) a mass analyzer, typically a time-of-flight (TOF) mass spectrometer which is designed to allow a fast acquisition rate and large mass detection range.
4) an ion detector.

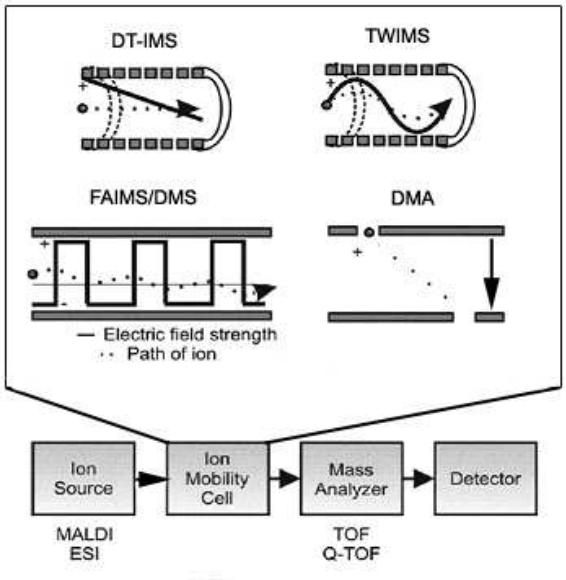

***FIGURE* 2.** Overview of typical ion-mobility mass spectrometer configurations, adapted from Enders and Mclean, 2009.

There are four main types of IM cell utilised in IMS-MS.

(i) Drift-time IMS (DT-IMS) is the simplest configuration where collision cross-section (CCS) can be directly calculated without calibration and provides the highest resolving power. A tube is filled with a buffer gas (or mixture) and a low voltage field is applied, typically from 5 to 100 Vcm^{-1}. The ions collide with neutral buffer gas molecules, exit via a detector and the collision cross-section Ω_T, at a temperature T can be obtained by measuring the velocity of the ions and solving the Mason-Schamp equations at intermediate electrical fields (5-100 Vcm^{-1}):

$$V_d = KE \tag{3}$$

where V_d is the drift velocity of the ion, K is the ion mobility, E is the imposed electric field, and

$$\Omega_T = \left(\frac{3ze}{16N}\right)\left(\frac{2\pi}{\mu kT}\right)^{1/2}\left(\frac{1}{K}\right) \tag{4}$$

where z is the numerical charge, e is the elementary charge, N is the number density of the buffer gas, μ is the reduced mass of the ion- buffer gas neutral pair, k is the Boltzmann constant and T the temperature in Kelvin.

DT-IMS suffers from an inherent lack of sensitivity, due to a pulsed analysis (where ions are measured in packets), and the subsequent loss in duty cycle, as the time between packets of ions is not utilised. A review of IMS by Eiceman and Karpas (2004) discusses the history of IMS, the chemistry and physics of ion behaviour and reflects on the potential future development and applications of IMS.

In linear DT-IMS, illustrated in FIGURE 3, the sample is introduced to an ionisation region where ionisation can take place by a number of methods including β-emission from a ^{63}Ni corona discharge, photo-ionisation, electrospray etc. Ions are allowed through an electric shutter grid, whilst neutrals remain in the ionisation source and the measurement time is initiated. The drift tube can vary in length from 5 centimetres to 3 metres or more. An electric field gradient, typically from 10-100 Vcm^{-1}, from the ionisation source to the detector causes the ions to traverse the drift tube at a constant velocity. A drift gas is introduced counter-current to the flow of ions keeping the drift-tube free of neutrals which could participate in ion-neutral clusters.

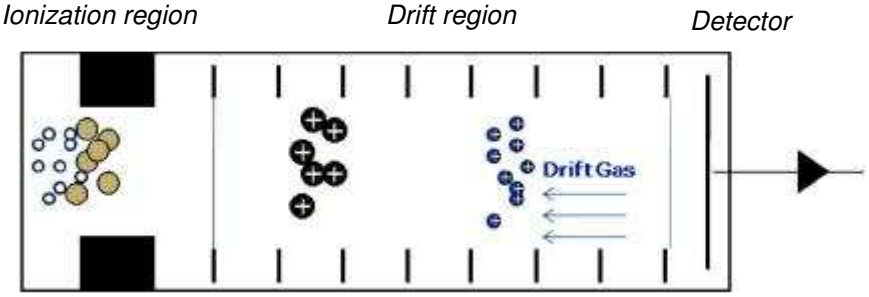

FIGURE 3. Illustration of DT-IMS, adapted from Eiceman and Karpas (2004). A voltage gradient is applied to the ions from left to right.

The total ion signal is detected and plotted with respect to time to form an ion mobility spectrum, e.g. (see FIGURE 4) for a mixture of amphetamines analysed by ESI-DT-IMS-MS (Matz and Hill, 2002). Smaller ions travel faster through the drift region and have shorter drift times, compared to higher molecular weight ions that drift slower and possess longer drift times.

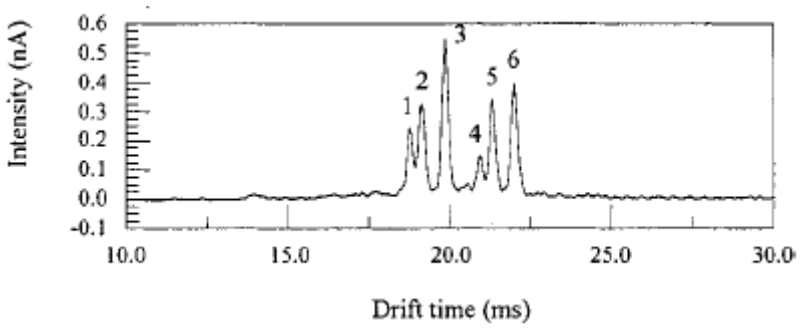

FIGURE 4. ESI-DT-IMS-MS spectrum of a mixture of 1) amphetamine, 2) methamphetamine, 3) ethylamphetamine, 4) 3,4-methyldioxyamphetamine, 5) 3,4-methylenedioxy methamphetamine and 6) 3,4-methylenedioxyethylamphetamine, adapted from Matz and Hill (2002a).

Another complementary approach to classical DT-IMS-MS was the use of the second quadrupole (Figure 5) in a triple-quadrupole mass spectrometer as an ion mobility device; while this approach was never commercialized it did open up opportunities in hyphenation of IMS and MS. In the triple quadrupole energy loss method an incident ion is transmitted to the second quadrupole of a triple quadrupole mass spectrometer where the ion will experience a drag coefficient and generate a stopping curve; from the stopping curve the ion mobility can be measured and a collision cross-section determined. Indeed, Covey and Douglas (1993) were the first to measure collision cross- sections for some biomolecules using this method and also later reviewed collision dynamics in quadrupole systems including an assessment of the internal energy of the $C_6H_5^+$ ion by measuring the increase in collision cross-section after collisional activation (Douglas, 1998) .

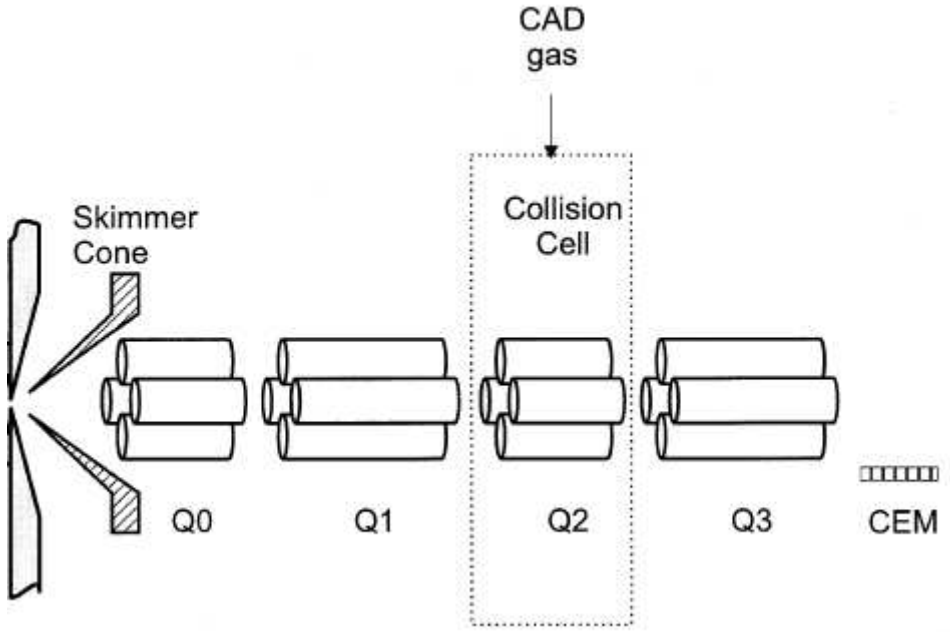

FIGURE 5. Schematic of the triple quadrupole configuration that can be used to obtain ion mobility measurements via an energy loss method, adapted from Purves et al. (2000).

This triple-quadrupole energy loss method was further developed to utilise segmented collision cell rods separated by small 1 mm gaps that enabled a radio-frequency only quadrupole drift cell to be used to reduce ion losses due to diffusion and enable mass selection before or after the drift cell, and a DC gradient that moved the ions in an axial direction (see Figure 6, Javahery & Thomson, 1997). Unfortunately this configuration was outside the typical low-field IMS range and may have resulted in field-heating of ions. This configuration was later improved to possess an increased gas pressure and lower field concentration that resulted in minimal internal excitation of the ions (Guo et al. 2004).

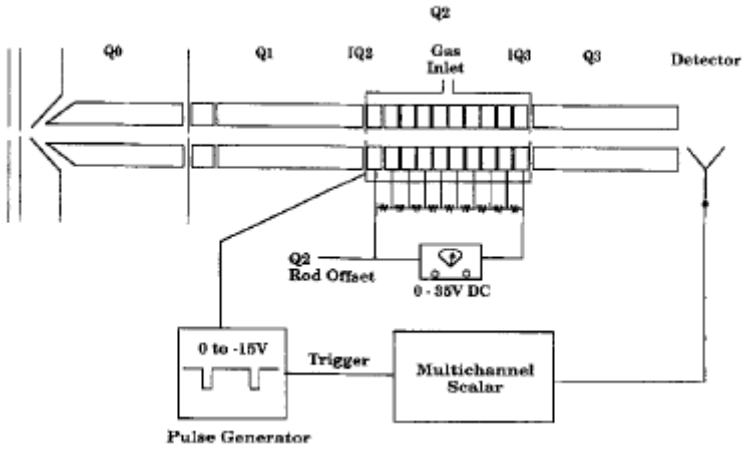

FIGURE 6. Schematic of a segmented triple quadrupole configuration that can be used to obtain ion mobility measurements, adapted from Javahery & Thomson (1997).

(ii) Differential mobility IMS (DMS) or FAIMS uses a sequence of intermediate and high field regimes where the behaviour of ions is described empirically by the Mason-Schamp equation (Mason and McDaniel, 1988) under a high-field regime, which can be expanded to an infinite series of E/N:

$$K_0(E) = K_0(1 + a(E/N)^2 + b(E/N)^4 + c(E/N)^6 + ...) \quad (5)$$

where K_0 is the reduced ion mobility, E is the field intensity and N is the buffer gas number density. However, under a typical FAIMS electric field the mobility can be approximated by using the first two factors as the importance of the sixth order and higher are insignificant (the fourth order is two orders of magnitude smaller than second order, and the second order is three to five orders of magnitude smaller than one (see Shvartsburg et al., 2004).

The basic principle of operation is that ions are introduced to a region with electrodes and a stream of gas acts as a transport medium. An asymmetric waveform is passed across the electrodes, which consists of a high potential electric field for a short time followed by a low potential electric field for a longer time; this typically fixed dispersion voltage (DV) waveform is superimposed with a variable compensation voltage (CV) to maintain a stable trajectory for the analyte ion. This process will effectively select ions and act as an ion filter, as shown in Figure 7. A cylindrical electrode configuration has usually been designated FAIMS, whereas parallel plate configurations have typically been designated as DMS.

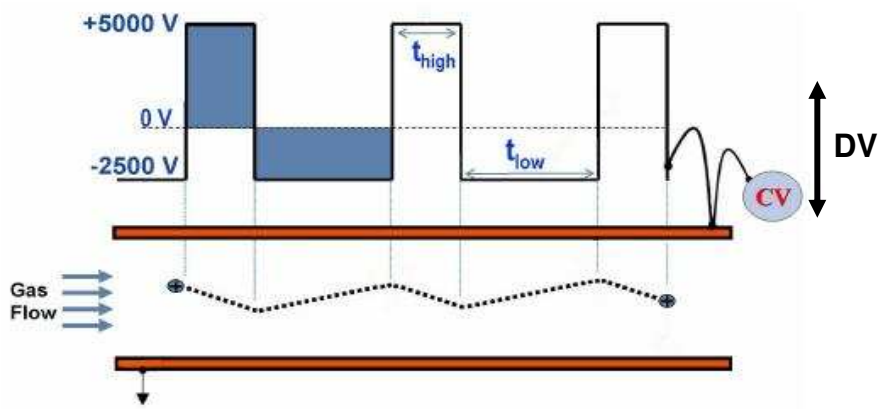

FIGURE 7. Illustration of an example of a parallel plate DMS with compensation voltage applied showing ion transmission, adapted from Thermo Fisher Scientific Inc., FAIMS Operators manual (2007).

A compensation voltage scan measurement (Figure 8) shows a typical profile attained by optimising the effect of inner and outer electrode temperatures on peak profile for morphine and its 3-β-D-glucuronide metabolite (Hatsis et al., 2007). Optimising the separation of morphine whilst maintaining sensitivity was reported to enable the metabolite interference to be effectively filtered out, and significantly improve the quantification of morphine.

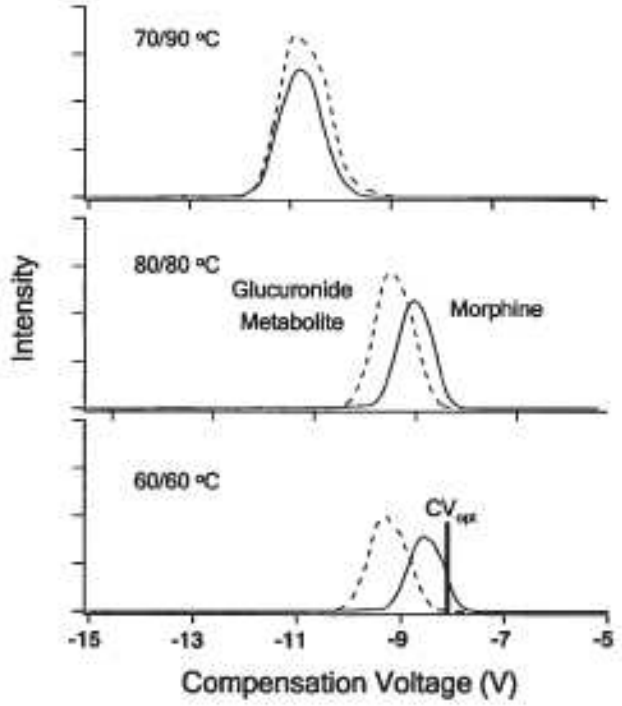

FIGURE 8. Compensation voltage scans of morphine and the glucoronide metabolite, adapted from Hatsis et al., (2007).

The use of compensation voltage optimisation to select an analyte ion can result in a clear difference in the mass spectrum observed resulting in an increased S:N ratio for the analyte and a reduction in other signals as shown in the use of ESI-FAIMS-MS with cisplatin and its hydrolysis products (Cui et al., 2003) shown in FIGURE 9.

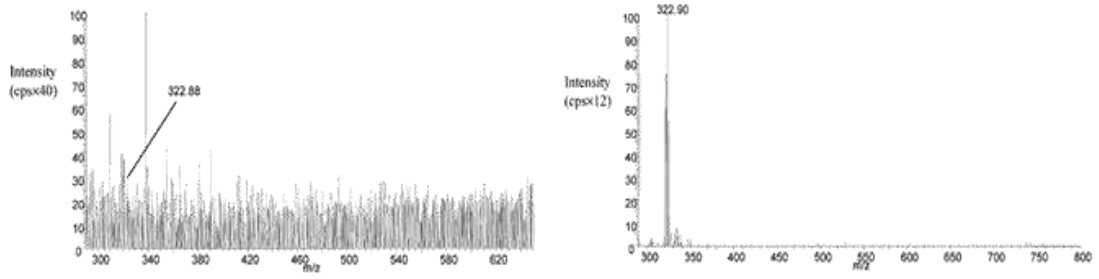

FIGURE 9. Mass spectra showing the reduction in background noise from (a) ESI-MS to (b) ESI-FAIMS-MS, adapted from Cui et al. (2003).

Currently the factors determining the separation mechanism in FAIMS or DMS and factors governing peak width as well as transmission remain relatively difficult to predict (Shvartsburg et al., 2004). The parameters influencing performance include field intensity, ion path length, gas properties (composition, temperature, pressure), shape and width of electrodes, the profile and frequency of asymmetric waveform, compensation voltage scan speed and gas flow. Champarnaud et al., (2009) studied the separation of trace level impurities by combining experimental observation with a Design of Experiment (DOE) statistical treatment that indicated important factors in the optimisation of the values of the compensation voltage, signal intensity, separation, peak asymmetry and peak width. However, a study of tetraalkylammonium ions found standard conditions were often suitable for selecting ions with an m/z value of 100-700 (Aksenov and Kapron, 2010). The simulation of ion motion in planar electrode FAIMS and cylindrical electrode FAIMS, provided insights into design, experimental variables and interpretation (Smith et al., 2009b) and some of the key molecular and instrumental parameters affecting performance were discussed by Nazarov (2006) and Levin et al. (2004).

Whilst there are several novel geometries (Prieto, 2011), there are two main forms of field asymmetrical waveform ion mobility spectrometers: 1) those with planar electrode geometry and 2) those with curved electrode geometry. Both planar geometry and the curved geometries of FAIMS and DMS evolved during the 1990s, and in the early 1990s the term differential mobility spectrometry (DMS) became generally synonymous with planar electrodes and the term FAIMS became synonymous with curved geometries, although there are multiple examples of overlapping usage of the terms DMS and FAIMS. One of the primary differences relates to the use of polar transport gas modifiers. Planar devices create homogeneous electric fields which enables the use of transport gas modifiers without resolution losses whereas curved geometries tend to create inhomogeneous fields which lead to a loss in resolution when using transport gas modifiers. However, curved geometries have been shown to provide some degree of ion focusing at atmospheric pressure resulting in higher sensitivity (Guevremont & Purves, 1999; Krylov, 1999). Planar line-of-sight analyzers enjoy the convenience of transmitting all ions when the RF voltages are turned off, so that operation without mobility separation can be achieved simply by turning off the fields. Curved geometry/non line-of-sight FAIMS require the device to be removed for operation without mobility separation. A wider analysis of the differences between alternative geometries was described by Krylov (2003).

A comprehensive review discussed the applications of FAIMS for drinking water analysis, pharmaceutical metabolite identification and separation of isomers and isobaric peaks (Kolakowski and Mester, 2007). In addition a detailed account of the fundamentals of DMS and FAIMS has been written by Shvartsburg (Shvartsburg, 2010).

(iii) Travelling wave ion mobility spectrometry (TWIMS) is a novel method whereby ions are separated according to their mobility in a series of voltage pulses in a travelling wave (T-wave) mobility cell utilising RF ion guides (Gerlich, 1993). The resolving power is relatively low; however, collision cross-sections can be derived by calibration with known standards. This ion mobility approach has been successfully interfaced to a conventional time of flight mass spectrometer and, due to the trapping gates and fast data acquisition rate of the TOF, good sensitivity is achieved. Despite attempts (Shvartsburg and Smith, 2008; Smith et al., 2009a)

the motion of ions in TWIMS is not fully understood and TWIMS calibration is typically used to calculate CCS values. The commercial technical and software support has arguably reinforced attempts to utilise IMS-MS in separation, characterization and measurement applications, for example, via the routine use of multidimensional data in Driftscope software (Williams et al., 2009a) and software to process complex data such as time-aligned parallel fragmentation (D'Agostino and Chenier, 2010).

In travelling wave ion mobility spectrometry a transient DC voltage pulse is applied in order to create an electromotive force via a series of sequentially opposite polarity RF-only rings to create a travelling wave which propels ions through the device, as shown in Figure 10. Ions with high ion mobility slip behind the wave less often (or spend more time surfing) than ions of low ion mobility thus enabling separation based on relative ion mobility.

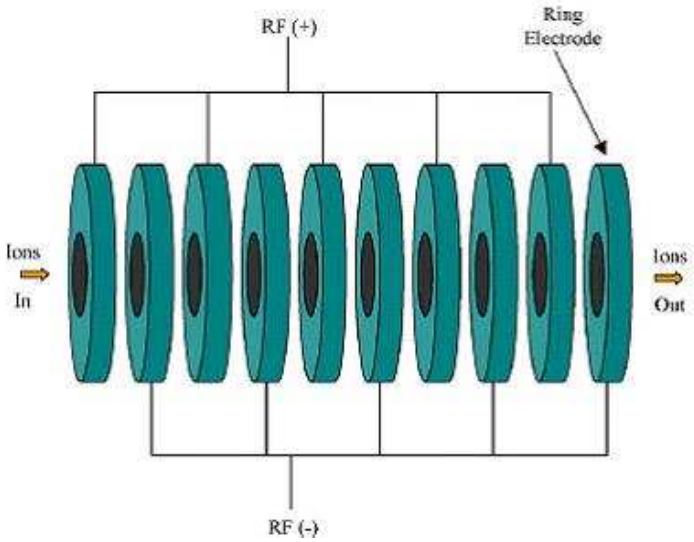

FIGURE 10. Illustration of a stacked ring ion guide used in traveling wave ion mobility spectrometry (TWIMS), adapted from Pringle et al. (2007).

Visualising data obtained using the proprietary Driftscope software enables a range of options for understanding the data including 3D visualisation, 2D plots and intensity views etc, The data can be interactively processed if desired, for example, to show data that only contains a certain component using various geometric selection tools such as lasso and square area.

(iv) The Differential Mobility Analyser (DMA) was originally developed to generate particles in order to calibrate aerosol instruments, later expanded to describe the mobility of non-diffusing particles by Knutson and Whitby (1975) and recently to describe the mobility of diffusing particles by Stolzenberg & McMurray (2008). The DMA consists of a combination of electric field mobility in addition to a fast gas stream, only ions with a well defined electrical mobility are transmitted into an outlet slit leading to the mass spectrometer inlet (see Figure 11). The DMA vacuum regime means that the measured ions do not experience a vacuum interface or ion guide so may be less prone to structural modifications (Hogan et al., 2011) and, as the separation technique is a space-dispersion rather than a time-dispersion technique, the ions can be continuously transmitted to a mass spectrometer.

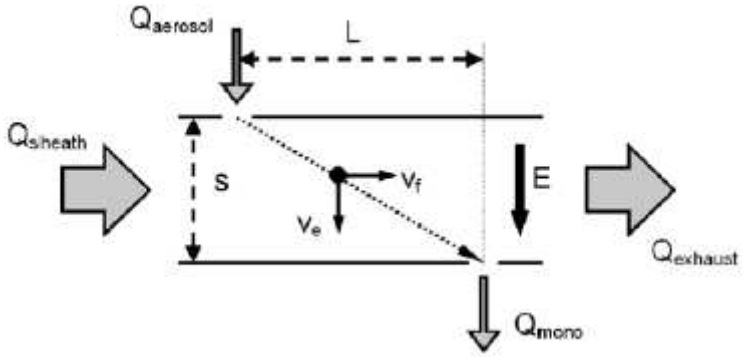

FIGURE 11. Schematic of the operation of a differential mobility analyzer. Ion are injected at the top left and move downwards and to the right, over a distance S, under the influence of an electric field E, adapted from de la Mora et al., (2006)

The DMA technique may be considered a hybrid of DT-IMS and DMS as the separation process is based on the low electric field mobility like DT-IMS, however the sampling is continuous as in DMS. DMA-MS has been most widely explored for large molecules; however, discussion on multiple charged polyethylene glycol ions (Ude et al. 2004) illuminated structures from approximately 300 Da to 3000 Da describing configurations for long straight chain molecules. One of the advantages of DMA is that it can, theoretically, be easily added to existing mass spectrometry stages without complex interfaces due to operation at atmospheric pressure (Rus et al., 2010). A DMA was coupled to an existing Sciex QStar MS (Concord, ON, Canada) enabling separation of L-alanine and an isomer, sarcosine, (Martínez-Lozano P et al., 2010) which are proposed to be small molecule biomarkers from urine in the progress of prostate cancer.

IMS covers a range of different techniques and unfortunately some gross simplifications have resulted in terminology that may be confusing.

1. DTIMS is also known as Classical IMS, Conventional IMS, Standard IMS, Drift-Tube IMS, Time of flight IMS, Traditional IMS, Plasma Chromatography and Ion Chromatography.
2. DMS includes High Field Asymmetric Waveform IMS (FAIMS), Field Ion Spectrometry and Ion mobility Spectroscopy and is commercialised in the Ionalytics Selectra, Thermo FAIMS, Owlstone Nanotechnologies and AB Sciex Selectra systems.
3. Travelling wave ion mobility spectrometry (TWIMS) is commercialised in the Waters Synapt systems.
4. Differential Mobility Analysers (DMA) have been developed by several groups at Yale (USA), CIEMAT (Madrid) and RAMEM (Madrid).

Comparison of key benefits and challenges of IMS-MS methods

There is currently a wealth of IMS-MS systems available both commercially and being used and developed in academic institutions.

DT-IMS-MS has been most widely used in academic institutions and provided some of the highest resolving powers. An advantage of DT-IMS-MS is that the ion mobility can be determined experimentally and collision cross section determined without requiring calibration. A key challenge is that the pulsed analysis leads to an inherent loss of duty cycle and hence reduction in sensitivity.

An advantage of both DMS-MS and FAIMS-MS is that it operates as a continuous device when the compensation voltage is selected so does not have the 'lossy' sampling issues of DT-IMS and TWIMS. The separation appears to be orthogonal to m/z and sometimes size so that separations may be uniquely tuned to select a chosen analyte and thus be used as a powerful separation technique. A key challenge is that it has proven difficult to definitively assign structural properties and changes to DMS measurements as several factors appear to contribute to the clustering/declustering mechanism and analyte drift times thus it appears to be best utilized as a separation device.

An advantage of TWIMS-MS is that it is commercially supported and that the ion mobility can be determined experimentally and collision cross-section determined with suitable calibration. Whilst the

resolution obtained in TWIMS is typically lower than dedicated DT-IMS-MS and some FAIMS/DMS the software to interpret and process the complex data is well supported.

Advantages of DMA-MS include operation in the low electric field regime that typically means less structural distortions and determination of collision cross-section, a continuous sampling rate that should mitigate sensitivity losses (with reported transmission efficiencies of up to 50% (Martínez-Lozano et al., 2011)) and the theoretical ability to add the device as a front-end to many existing mass spectrometer systems. However DMA-MS has not been fully commercialized yet or utilized for the multitude of "small molecule" applications explored in DT-IMS-MS, FAIMS-MS, DMS-MS and TWIMS-MS though it shows significant promise.

C. Understanding IMS-MS resolving power and selectivity

IMS can separate analytes based on their ion mobility including closely related species such as isomers (Williams et al., 2010), isobars and isotopomers (Shvartsburg et al., 2010a). The key parameters affecting a useful separation are 1) the resolving power and 2) the selectivity.

Resolution and peak capacity in IMS

The combination of ion mobility and mass spectrometry in IMS-MS offers a technique that is able to distinguish components based on their size to charge ratio (Ω/*z* for IMS) and mass to charge ratio (*m/z* for MS), thereby enabling orthogonal specificity. Even with expensive high-resolution mass spectrometer systems affording $m/\Delta m_{50\%}$ resolution of over 400,000 it is still analytically challenging to differentiate between isomeric components and often complex MS^n experiments are required to achieve selectivity for unambiguous assignment. Ion mobility can provide extra resolving power, however IMS used alone is currently unable to unambiguously identify an unknown molecular component without *a priori* knowledge of the measured drift time.

It is possible to measure the resolving power of ion mobility using a single quotient definition (Siems et al., 1994):

$$R = dt / wh \qquad (6)$$

where *R* is the resolving power of the IMS, *dt* is the drift time of the ion of interest and *wh* is the full peak width measured at half height. Resolving power is a measure of the efficiency of an instrument to separate two peaks. The Waters Synapt G2 IMS system has been developed to encompass a resolving power of up to 40; FAIMS resolving powers of up to 100 (Shvartsburg et al., 2010b) have been achieved, and several reports of resolving powers of up to 225 with DT-IMS have been reported (Koeniger et al., 2006; Shelimov et al., 1997; Kemper et al., 2009). Developments including higher pressure trapping and focussing (Clowers et al., 2008), overtone ion-mobility (Valentine et al., 2009) and circular instruments (Bohrer et al., 2008) are expected to exceed these current limitations.

The number of theoretical plates is a mathematical concept, relevant in any chromatographic technique, which is often used to describe column efficiency and is an indirect measure of the peak width for a peak at a specific retention time:

$$N = 5.545\left(\frac{t_R}{W_h}\right)^2 \qquad (7)$$

where N = number of plates, t_R = retention time and W_h = peak width at half height (in units of time). The number of theoretical plates is typically used to compare chromatographic systems and the data in Table 2 compares various types of IMS with typical traditional chromatographic techniques.

TABLE 2. A comparison of required resolving power in theoretical plates for various types of IMS compared to typical chromatographic conditions.

Approximate number of theoretical plates	Required resolving power of equivalent	Comparative chromatography conditions

	IMS	
20000	60	High performance liquid chromatography (HPLC)
80000	120	Ultra high performance liquid chromatography (UHPLC)
125000	150	Gas chromatography (GC)
222000	*200*	*High resolution IMS (Asbury & Hill, 2000a)*
887000	400	Capillary electrophoresis (CE)

Therefore, a current high-resolution IMS resolving power of 200 is roughly equivalent to a chromatographic efficiency of >200,000 theoretical plates.

Peak capacity of IMS-MS

Complex samples require high efficiency to achieve separation and, even in early stage drug discovery, having a high efficiency affords a good opportunity to separate degradants and process impurities away from the desired product. Peak capacity is defined as the maximum number of peaks that can fit in any two-dimensional method (Ruotolo et al., 2002a). A two-dimensional method will have a high peak capacity if the resolution of each dimension is high and the difference in their separation mechanism (orthogonality) is high. The peak capacity will also be defined by the complexity of the sample and the properties of the analytes in the sample. For example, complex biological samples with a range of retention times will provide larger peak capacities for a technique than that of the analysis of a mixture of analytes of a specific class. Peak capacity is therefore a better, but highly molecule dependent, indicator of the separation power compared to measuring resolution alone.

In an ideal situation the peak capacity of a two-dimensional method is the product of the first and second dimensions (Li et al., 2009), but corrections can be made for cases where the two dimensions are not 100% orthogonal. Whilst the separation based on their size to charge ratio (*Ω/z* for IMS) and mass to charge (*m/z* for MS) ratio is, to some extent, orthogonal there is a well-known correlation between mobility (∝ size) and mass (Griffin et al., 1973), as illustrated in Figure 12.

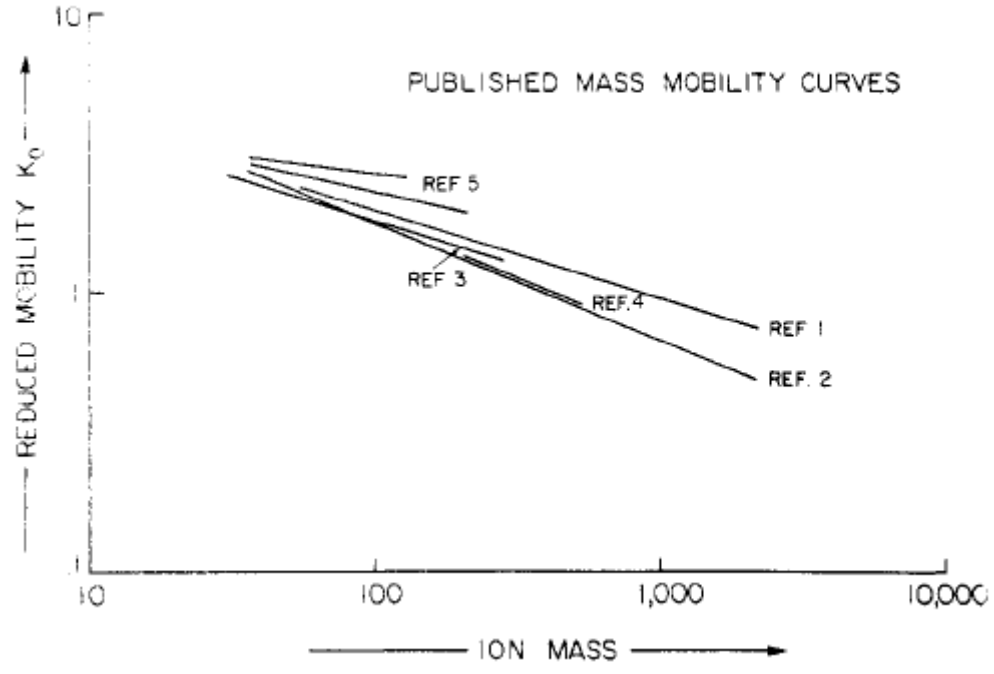

FIGURE 12. Published mass mobility curves showing correlation between reduced mobility and mass within classes, but poor correlation in heterogeneous sets, adapted from Griffin et al. (1973).

Therefore the corrected peak capacity, P_c, in IMS-MS can theoretically be estimated (Dwivedi et al., 2010) using the relationship:

$$P_c = R_{IMS} \times R_{MS} \times \text{fraction of orthogonality} \quad (8)$$

where R_{IMS} is the average resolving power of the ion mobility spectrometer, and R_{MS} is the average resolution of the mass spectrometer.

An example of increased peak capacity in IMS-MS was observed in a study of various classes of metabolites in blood (Dwivedi et al., 2010) including amino acids, organic acids, fatty acids, purines etc) using a DT-IMS-MS system. In the mass range of 23 – 830 m/z, the drift time spread of ~14.3 ms results in ~28% of the total 2D space, or an average deviation in drift time of ±14% along the theoretical trend. With an average IMS resolving power of 90, average MS resolution of 1500, and ±14% orthogonality, the estimated peak capacity, P_c, for the instrument is 90 x 1500 x 14% = 18,900. The relatively low MS resolution of 1500 (peak width is 0.27 Da at an average mass value of 404 m/z) in this study (Dwivedi et al., 2010) resulted in an estimated peak capacity of MS alone of 2989. A six fold increase in the peak capacity was therefore observed (~19,000) in IMS-MS compared to MS alone (~3000).
Reverse phase chromatographic columns are routinely used to separate small molecules and the peak capacity for gradient elution high performance liquid chromatography was found to be typically up to 300 (Guo et al., 2009), and up to 400 (Wren, 2005) for gradient elution ultra-performance liquid chromatography. Comparing the peak capacity for different types of IMS with typical traditional chromatography peak capacities (Table 3) shows that the extra dimension of IMS is potentially a powerful separation tool.

TABLE 3. Approximate separation peak capacity for various analytical separation methods*.

Technique	Approximate peak capacity
FAIMS	*8.9 -44 (Canterbury et al., 2008)(Schneider et al., 2010b)*
DT-IMS	*90 (Dwivedi et al., 2010)*
HPLC	300 (Guo et al., 2009)
UHPLC	400 (Wren, 2005)
MS	3000 (Dwivedi et al., 2010)
IMS-MS	*19000 (Dwivedi et al., 2010)*
LC-MS	900000
LC-IMS-MS	*11340000 (Dwivedi et al., 2010)*

*Ion mobility and hyphenated techniques are italicized

It is conceivable that LC-IMS-MS may become a standard addition or replacement for LC-MS systems due to the ease of configuration and the increase in separating power. Indeed many proteomic applications are increasingly using LC-IMS-MS to separate complex peptide mixtures, leading to unprecedented extensive proteome maps (Liu et al., 2007b; Taraszka et al., 2005). LC-IMS-MS often does not require careful configuration, demanding sampling rates and does not appear to suffer from robustness issues compared to many two-dimensional techniques such as LCxLC, GCxGC etc.

Modifying selectivity in IMS

The selectivity of ion mobility can be modified by increasing the electric field in DT-IMS (Wu et al., 1998), the use of covalent shift reagents (Fenn and McLean, 2008) to derivatise or non-covalent shift reagents (Clowers and Hill, 2006; Howdle et al., 2009) to form complexes with analytes and effect a selective shift in ion mobility relative to coincident analytes, using drift gas modifiers (Fernández-Maestre et al., 2010a) sometimes called clustering agents in DMS-MS (Schneider et al., 2010b)), by altering the composition of the drift gas (Matz et al., 2002) and by the use of different reagent gases in GC-IMS-MS (Eiceman et al., 1995). Currently there does not appear to be a consensus on useful and well characterised selectivity modifiers so method development is presently not fully predictable; however some of these changes are trivial and can be considered analogous to changing the stationary or mobile phases in liquid chromatography.

The use of covalent shift reagents (Fenn and McLean, 2008), as shown in Figure 13 , effectively derivatises molecules, potentially creating lower density analogues of the precursor species with a marked increase in the collision cross-section relative to a smaller increase in mass. This is clearly

illustrated by deviations to larger cross sections compared to the general trend in mass-mobility correlation for carbohydrates. Compared to the underivatised species it was reported that covalent derivatisation afforded three distinct advantages: (i) tuneability was increased for isobaric species difficult to identify and/or resolve by mass spectrometry alone, (ii) an enhanced sensitivity of 2x more signal intensity was observed and (iii) the derivatised species could be used as tags or fragment labels in CID and as IR active species in IRMPD studies.

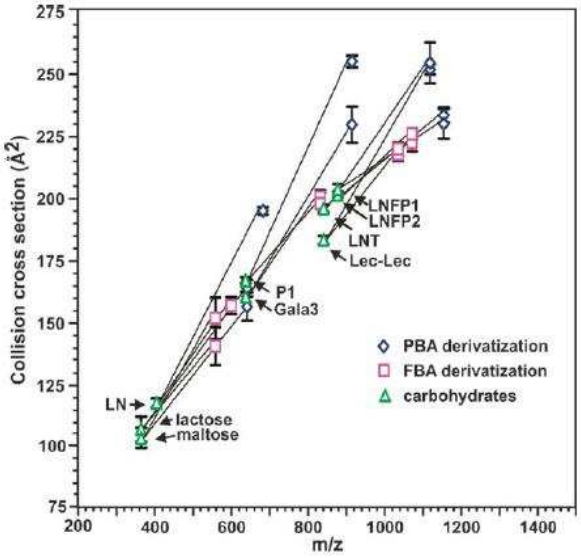

FIGURE 13. Effect of derivatisation of carbohydrate species with boronic acid on CCS, adapted from Fenn and McLean (2008).

For small molecules the effect of the dipole interaction is typically far more significant than for large molecules (>500 Da) so that using drift gas modifiers or mixtures can be a powerful method to alter selectivity (elution order of analytes). Indeed for small molecules such as amino acids the polarisabiity has been found to be a critical factor affecting separation of analytes, whereas in large molecules the collision cross section term dominates (Steiner et al., 2006). Thus for small molecules exploiting polarizability to probe structural details and maximize separation has immense future potential.

An example of the potential of exploiting polarizability to separate analytes is given by the different slopes of calculated ion radii for iodoaniline and chloroaniline with different drift gases, helium (0.205 x 10^{-24} cm^3), argon (1.641 x 10^{-24} cm^3) and carbon dioxide (2.911 x 10^{-24} cm^3), indicating that it should be possible to separate any analytes with different slopes (Figure 14) by choosing an appropriate drift gas composition.

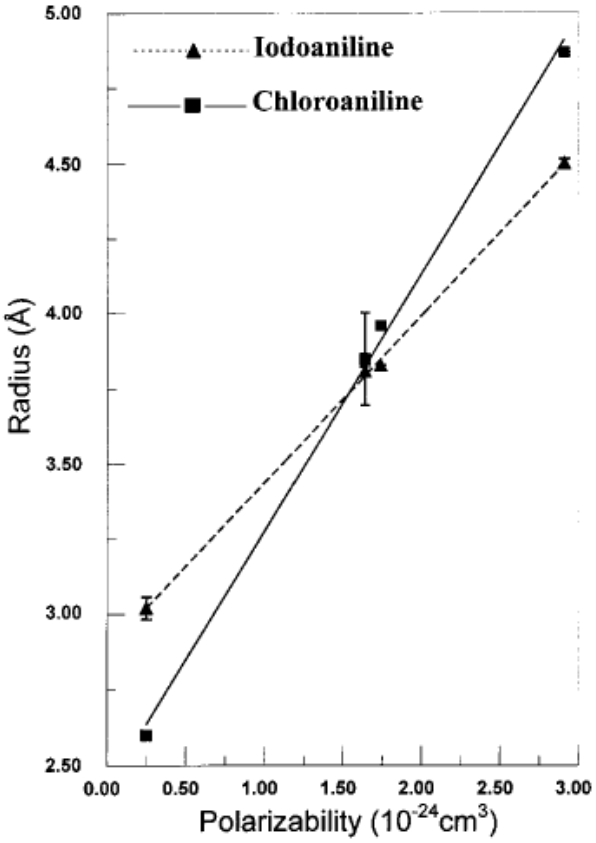

FIGURE 14. Calculated ion radii as a function of drift gas polarizability, from Asbury & Hill, 2000c.

The use of vapour modifiers added to the drift gas in planar DMS-MS has been widely explored (e.g. Levin et al., 2007 and Eiceman et al. 2004) , although the full mechanism of the interactions has not been elucidated enough to enable predictable separations in mixtures. Levin et al. (2004) systematically examined the effect of various polar clustering agents and postulated strong effects due to hydrogen-bonding potential, electrostatic attraction, steric repulsion and energetically feasible conformations. A series ofpublications exploring the cluster/declustering (Krylov et al. 2002, Krylov et al. 2009, Schneider et al. 2010a, Schneider et al. 2010c, Coy et al. 2010) further explored the possibility of predicting analyte shifts in response to changes in drift gas modification and provide a powerful route to optimising separations.

The 'cluster/declustering effect' that addition of vapour modifier induces appears to have dramatic effects on the peak capacity in a planar electrode configuration (Schneider et al., 2010b), improving the peak capacity for a 70 component mixture from 13 in pure nitrogen to 44 in 2-propanol doped nitrogen (see FIGURE 15), although this had the disadvantage that 10/70 components were depleted in intensity by 20-fold or more due to them having a lower proton affinity than the dopant.

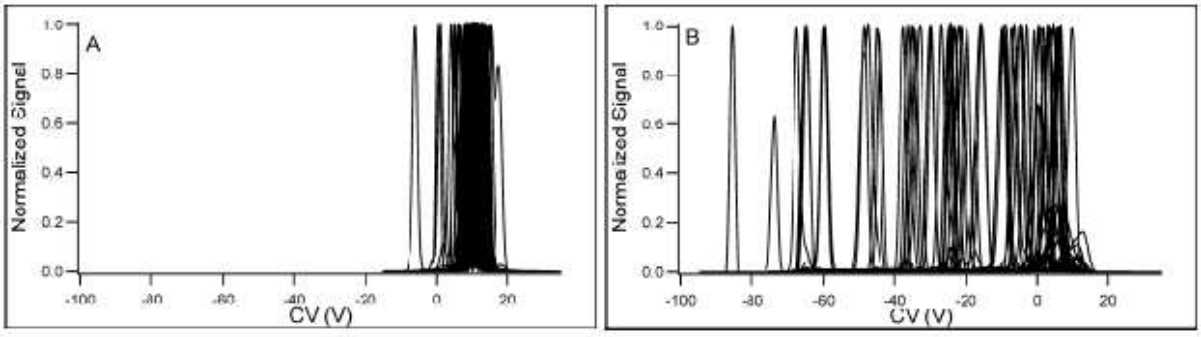

FIGURE 15. Separation of a 70-compound mixture with (A) nitrogen transport gas and (B) nitrogen with 1.5% 2-propanol in parallel plate FAIMS configuration, adapted from Schneider et al., (2010b).

Changes to the temperature, composition (Beegle et al., 2001) and pressure of a single drift gas is commonly used to change selectivity and IMS gasses including nitrogen, air, helium, carbon dioxide and sulphur hexafluoride have been evaluated. The use of binary gas mixtures (Howdle et al., 2010), shown in Figure 16, results in excellent selectivity enhancements over single gas composition IMS separations and demonstrates that this selectivity is tunable by altering the binary gas composition.

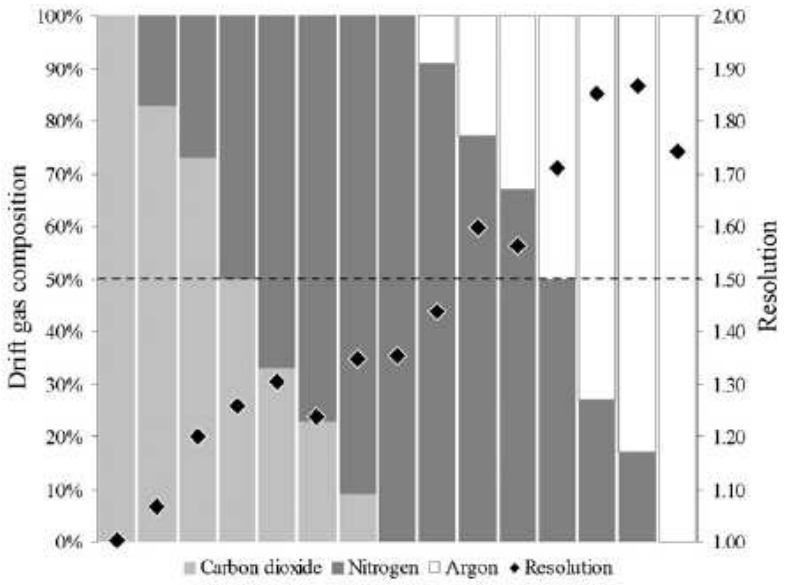

FIGURE 16. Effect of drift gas composition on the ion mobility resolution of the drugs rosiglitazone and lamotrigine $[M+H]^+$ ions in binary drift gas mixtures in TWIMS. The composition of the binary drift gas mixtures are represented by shaded bars indicating the percentage of each gas in the mixture (left hand axis). Resolving powers greater than 1.5 indicate full separation of components and this threshold is indicated by the dashed line at a resolution of 1.5. The resolution of lamotrigine and rosiglitazone is indicated by the right hand axis. Adapted from Howdle et al., (2010).

IV. APPLICATIONS OF IMS AND IMS-MS SEPARATIONS IN SMALL MOLECULE ANALYSIS

IMS has been investigated for the analysis of a wide range of small molecule applications including active pharmaceutical ingredients (Budimir et al., 2007; Karimi and Alizadeh, 2009; O'Donnell et al., 2008; Wang et al., 2007) veterinary drugs (Jafari et al., 2007), metabolites (Alonso et al., 2008), pesticides (Jafari, 2006; Keller et al., 2006; Tuovinen et al., 2000), prescription and illicit drugs (Dussy et al., 2008; Lawrence, 1986), combinatorial libraries (Collins and Lee, 2001), autonomous health diagnostics (Zhao et al., 2010) and immunoassay detection (Pris et al., 2009; Snyder et al., 1996). Here we outline some highlights of small molecule analysis using IMS-MS systems.

A. Low abundance metabolite and small molecule identification using IMS-MS

A novel use of LC-IMS-MS was demonstrated for a $5HT_3$ antagonist, ondansetron, and its aromatic hydroxyl isomeric metabolites (Dear et al., 2010) that are typically generated *in vivo* and *in vitro*. Using conventional UHPLC-MS-MS the unambiguous characterisation of the hydroxyl metabolites would not be possible as they can produce identical MS/MS spectra. Using UHPLC-MS in a biological matrix system ondansetron and metabolites display different retention times but could not be assigned without using purified standards as a reference. Using an IMS separation, shown in Figure 17, and *in silico* methods the components were identified based on their ion mobility. For these components a low number of rotatable bonds are present so the computational method is rapid, interpretation of complex NMR spectra is not required and isolation or synthesis is unnecessary to create primary standards. In this case the identity of metabolites with smaller than 1 $Å^2$ difference between their CCS was distinguished using a combination of Waters Synapt TWIMS by comparison with CCS values obtained using computational methods.

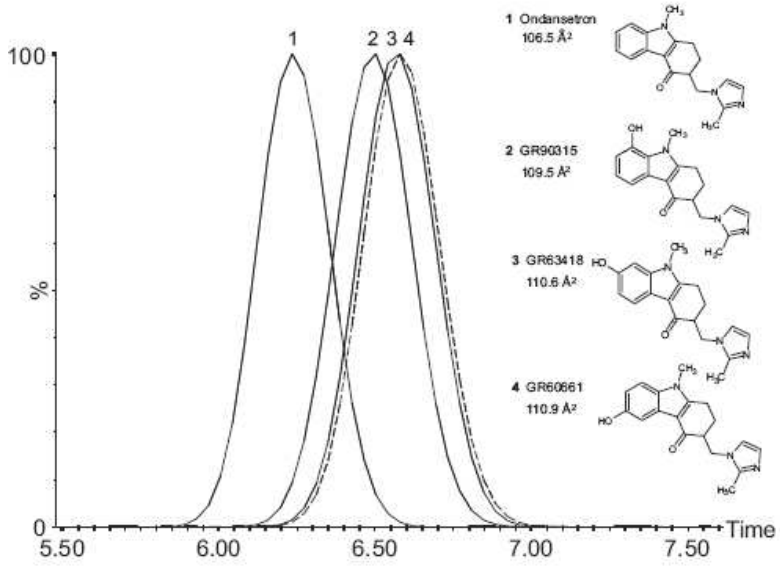

FIGURE 17. TWIMS ion mobility arrival time distributions for ondansetron and the 6-, 7- and 8-hydroxyl metabolites, adapted from Dear et al., (2010).

The subsequent use of product ion mobility as a tool for assignment of positional isomers (Cuyckens F et al., 2011) was also demonstrated for both model compounds and a real-case example, emphasizing the possibilities of structural determination by both parent ion and product ion mobility where mass spectra alone appear indistinguishable and cannot be used to confidently assign a candidate structure. For example two different product ion mobilities for 11 *ortho*, *meta* and *para* substituted hydroxyl metabolites with a phenylethylamine substructure were sufficient to assign their structures.

B. Rapid, portable and sensitive analysis using miniaturisation of IMS and IMS-MS

One of the advantages of FAIMS is that it does not require complicated vacuum equipment or large analyser tubes, thus it may be easily hyphenated to a portable mass spectrometer system (Manard et al., 2010). Microfabricated FAIMS chips can increase the speed of separation by 100-10,000 times, filtering ions on the microsecond timescale enabling rapid monitoring of species at low level concentrations (Shvartsburg et al., 2009a). Whilst the current microfabricated FAIMS units, example shown in Figure 18, are more suited to distinguish compound classes than individual species the multichannel FAIMS electrodes enables integration with an air sampler, ionisation source and detector for applications such as gas analysis, chemical monitoring and autonomous health diagnostics (Zhao et al., 2010).

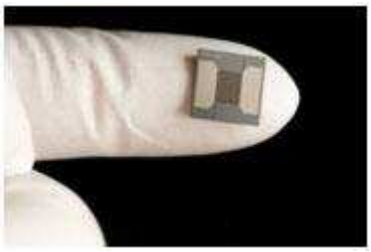

FIGURE 18. Illustration of the size of microfabricated FAIMS chip, courtesy of Owlstone Nanotechnologies and Pacific Northwest National Laboratory.

C. Increased selectivity in ambient and surface analysis mass spectrometry using IMS-MS

The direct and rapid analysis of substances using ambient ionisation mass spectrometry sources allows mass spectrometry data to be obtained with little or no sample preparation required for a variety of surfaces and matrices from tissue samples to intact tablet or liquid formulations. Application areas have included quantitative and qualitative measurements in pharmaceutical analysis, forensics, bioanalysis, *in vivo* imaging, proteomics etc. Whilst MALDI has been widely adopted in biological applications, for the analyses of biomolecules, there is currently a great deal of interest in ambient mass spectrometry approaches and there are now at least thirty methods documented (Weston, 2010).

The introduction of an additional IMS stage adds a further separation step to ambient mass spectrometry analysis without the need for rigorous sample preparation. Indeed for most surface analysis mass spectrometry methods the fact that the surface is sampled and ions are generated in a single step means that the only viable method of separation and selectivity before the mass detector is to use a gas-phase separation method such as IMS. This extra selectivity maybe particularly useful in imaging applications. In the case of the example of whole-body imaging of rats, the rats were dosed with 6 mg/kg *iv* with the anticancer drug vinblastine and the removal of interfering isobaric ions from endogenous lipids helps increase confidence in the MALDI imaging data (Jackson et al., 2007) by removing 'false positives' which, by mass spectrometry imaging alone, could be interpreted as containing a high concentration of the active drug, as shown in Figure 19. The extra dimension of separation could also prove useful in removing any matrix-related isobaric ions. The datasets from the Driftscope imaging platform were transferred to Biomap 3.7.5.5 for visualization enabling facile interpretation.

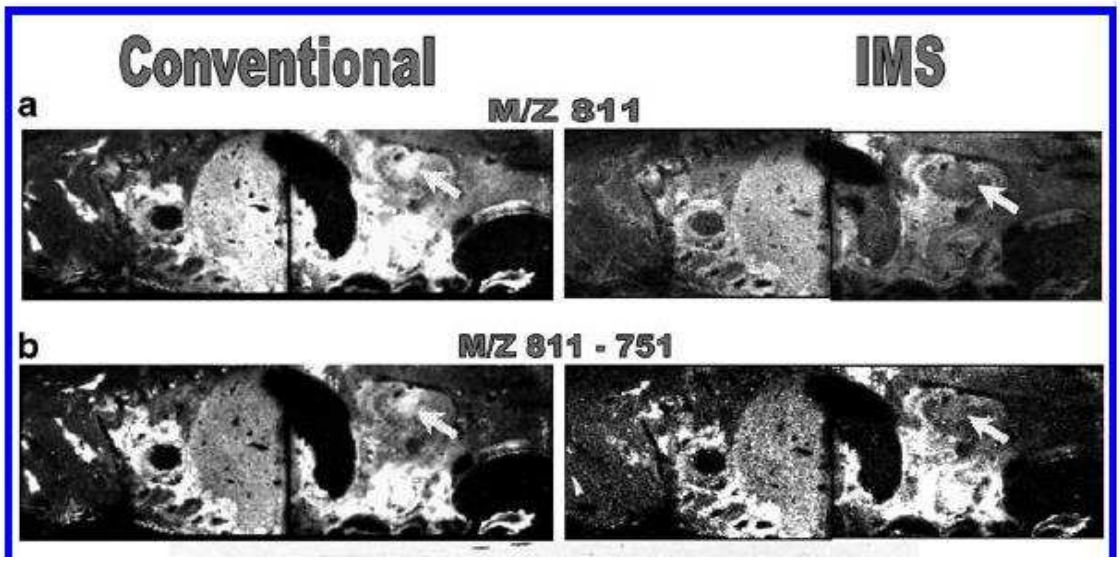

FIGURE 19 . MALDI-IMS-MS image showing distribution of ions in whole-body sections and the arrow points to the area where specificity increased with application of IMS, adapted from Jackson et al., (2007).

D. Chiral analysis using IMS-MS

A chiral modifier at 10 ppm of (S)-(+)-2-butanol was added to the buffer gas and enantiomers of a β-blocker, atenolol, were separated (Dwivedi et al., 2006), as shown in Figure 20. It is proposed that selective interactions occur in the gas-phase between the enantiomer ion and the chiral modifier, to temporarily form a diastereomeric pair, so that the mobilities of the enantiomers are altered and can be separated in time. Chiral ESI-DT-IMS-MS is now commercialised viathe Excellims Corp IMS-quadrupole-MS system. A smaller, portable chiral IMS detector is now being developed by Excellims Corp for fast, on-site analysis including pesticide residues and environmental samples (Anon). The advantages of chiral IMS-MS compared to competing analytical techniques such as chiral SFC and chiral HPLC include rapid method development and high sensitivity, enabling rapid determination of enantiomeric excess (e.e.) for use in QA/QC environments or in broader applications including biomarker and metabolite identification.

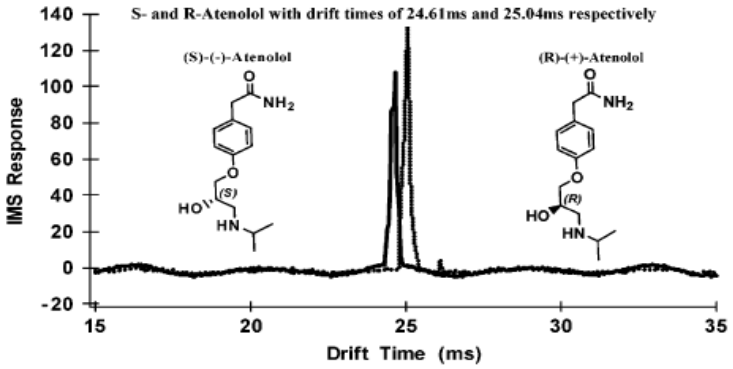

FIGURE 20. DT-IMS-MS separation of atenolol enantiomers showing the superimposed spectrum of the R and S enantiomers (similar results obtained using racemic mixture, not shown). Adapted from Dwivedi et al., (2006).

Chiral resolution using FAIMS-MS has also been reported for 6 pairs of amino acid enantiomers separated as metal-bound complexes of divalent metal ion with an L-form amino acid (Mie et al.,

2007), shown in Figure 21. The method employed a range of additional divalent metal cations and reference amino acids. Screening with different metal cations and reference compounds compares favourably with chiral HPLC and SFC screening times and can be automated using automated sample preparation platforms.

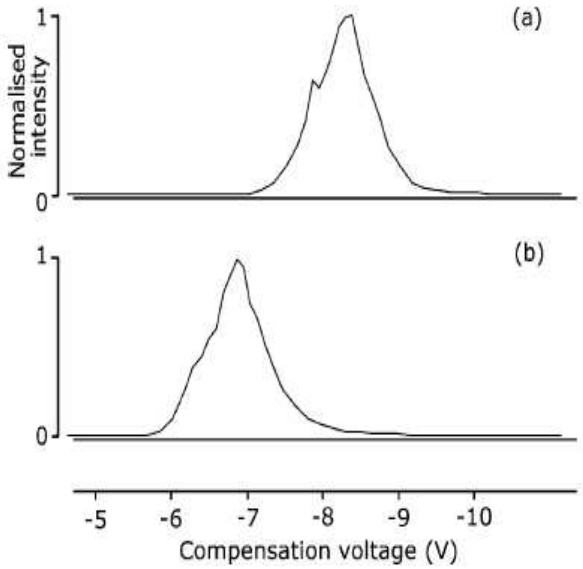

FIGURE 21. Separation of D/L-valine as $[Cu^{2+}(l\text{-}Trp)_2(D/L\text{-}Val)\text{-}H]^+$. (a) D-Val; (b) L-Val. Adapted from Mie et al., (2007) .

An example of epimer separation where the diastereomers differ by only one chiral carbon, was achieved for betamethasone and dexamethasone (Campuzano et al., 2011). The separation of the two epimers correlated well with differences observed in the calculated B3LYP/6-31G++(d,p) electrostatic potential surface. Whilst baseline separation is achievable by HPLC (Arthur et al., 2004) the mass spectra of these compounds is very similar so the rapid separation and correlation with molecular modeling quickly identify this pair of compounds.

E. Resolution of isobars and isomers in complex mixtures using IMS-MS

Over 1100 metabolites were detected from methanolic extracts of 50 ul of blood samples including separation of over 300 isobaric/isomeric components, achieved without pre-concentration (Dwivedi et al., 2010), shown in Figure 22. The peak capacity compared tomass spectrometric analysis alone was increased by ~6 times and a broad range of metabolites were detected including lipids, carbohydrates, isoprenoids and estrogens. Interpretation of the data is further enabled by examining characteristic mobility-mass correlation data to identify similar classes of metabolites. In addition a reduction in the background noise due to selective ion filtering enabled detection and identification of low abundance components.

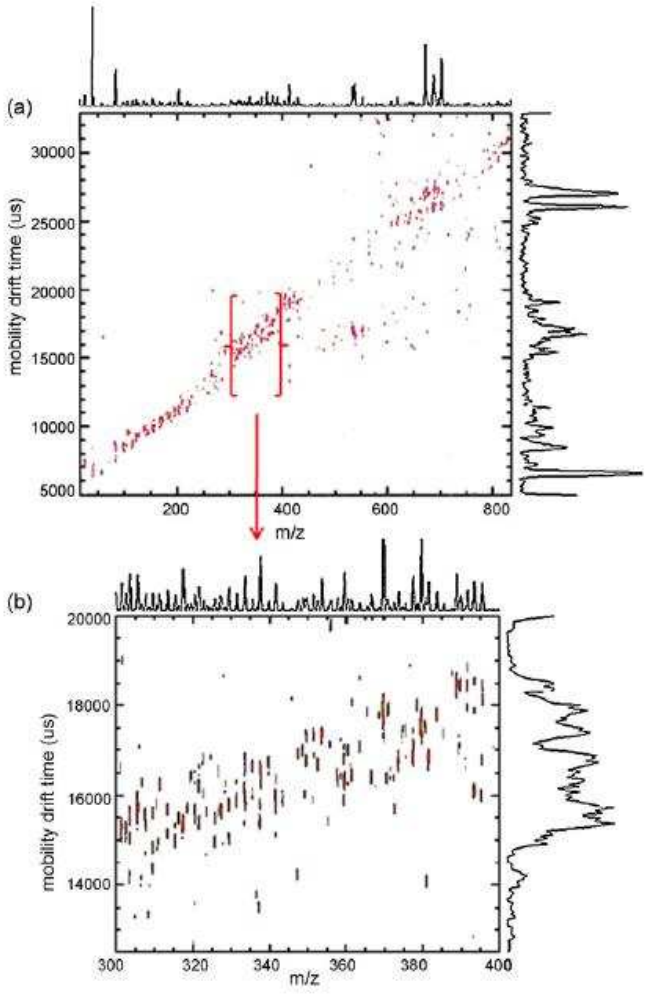

FIGURE 22. (a) Two-dimensional spectra of metabolic features measured in methanolic extract of human blood (b) a zoomed in region of the DT-IMS-MS spectrum illustrating peaks detected at the same nominal mass with different mobilities showing separation of isomers and isobars, adapted from Dwivedi et al., (2010).

F. Real-time reaction monitoring and process monitoring using IMS-MS

Reaction-monitoring in real-time has the potential to enable understanding of when reactions can be terminated at a suitable, rather than arbitrary, endpoint. By monitoring a process regularly throughout the reaction time knowledge may also be accrued of the reaction, intermediates and product formation that could not be understood by irregular, sparse sampling alone and enable optimization of experimental parameters via chemometrics. The products formed by deprotonation of 7-fluoro-6-hydroxy-2-methylindole with sodium hydroxide were monitored by TWIMS-MS (Harry et al., 2011) and showed complementary and extra information from TWIMS-MS compared to MS alone with shape selectivity information obtained by sampling every several minutes over a timescale of several hours (Figure 23).

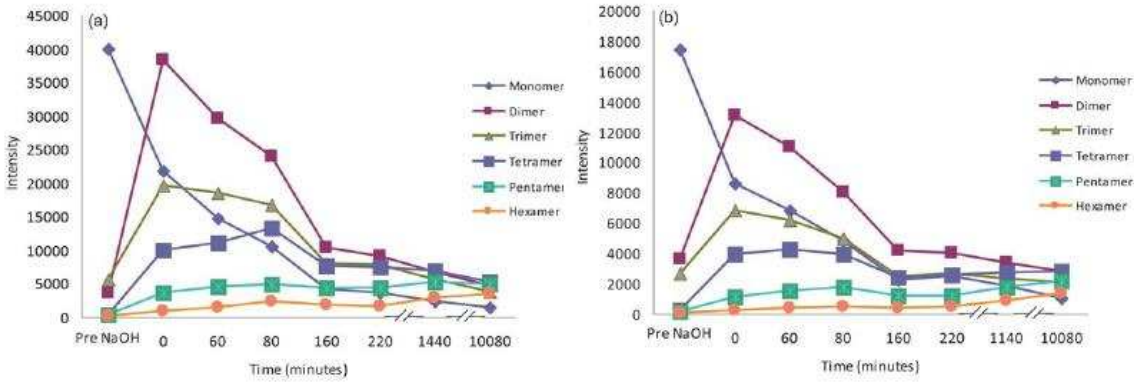

FIGURE 23. MS and TWIMS-MS analysis of the reaction of 7-fluoro-6-hydroxy-2-methylindole following the addition of aqueous sodium hydroxide. Signal response versus time in minutes for m/z 166 (monomer, I), m/z 311 (*O*-linked dimer, II), m/z 456 (*O*-linked trimer), m/z 601 (*O*-linked tetramer), m/z 746 (*O*-linked pentamer) and m/z 891 (*O*-linked hexamer) using (a) MS and (b) IM-MS. Adapted from Harry et al., (2011).

G. Rapid resolution of carbohydrate isomers using IMS-MS

Carbohydrate isomers including oligosaccharides are involved in numerous biological processes, such as cell-cell recognition and the development of embryos, but one of the main functions of carbohydrates is as oxidisable substrates in catabolism. However, to fully understand their different roles and functions we need to understand both the linkage type and anomeric configuration whilst dealing with the challenge that, for example, in a mixture of 16 D and L-aldohexoses and 8 D and L-aldoses the total number of isomers with the same mass will be 96. The use of mass spectrometry as a tool is hindered by the similarity between fragmentation data obtained for different isomers; however purification and determination of purity by NMR requires interpretation time and larger amounts of material. Separation of the metal ion adducts of anomeric methyl glycoside isomers (MeMan, MeGal and MeGlc) and isomeric forms of reducing sugars (Dwivedi et al., 2007), branch isomers, and very closely related isomers varying at a single stereochemical position (Zhu et al., 2009) were addressed where MS^n was not able to deliver solutions to the problem, as shown in Figure 24.

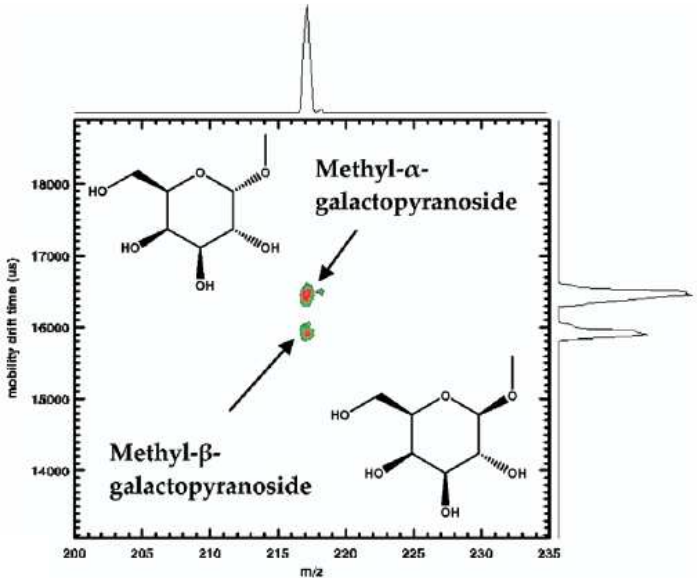

FIGURE 24. Two-dimensional DT-IMS-MS spectra of a mixture of methyl-α and β-D-galactopyranosides showing the separation (N_2 drift gas) of the sodium adducts at m/z 217, adapted from Dwivedi et al. (2007).

H. Rapid analyte testing in complex drug formulations by IMS-MS

The combination of IMS-MS with ambient ionisation mass spectrometry may enable rapid analysis for complex mixtures including drug formulations without laborious method development and consumables required by other separation methods such as 2D LC-MS etc.

The complementary techniques of IMS and DART ambient ionisation operated separately has been demonstrated for AG-013736 in 1 mg Axitinib tablets (Likar et al., 2011), enabling a rapid analysis of AG-013736 in AG-013736 drug substances by DART ionisation and analysis of low-level limits for absence of the drug in placebo tablets by ion mobility spectrometry using a Model 400B IONSCAN-LS from Smiths Detection Scientific (Danbury, CT).

Hyphenated ambient ionisation IMS-MS and nano-electrospray has been used to analyse:

- pharmaceutical formulations including tablets and creams containing one or more of ranitidine, paracetamol, codeine, anastrozole chlorhexidine and a nicotine-containing skin patch (Weston et al., 2005) using DESI.
- pharmaceutical formulations from tablets containing one or more of timolol, paroxetine, paracetamol and codeine using nano-electrospray ionisation (Budimir et al., 2007).
- pharmaceutical formulations containing one or more of paracetomol, ephedrine, codeine and caffeine from non-bonded reversed-phase thin layer chromatography (RP-TLC) plates by desorption electrospray ionisation (DESI) (Harry et al., 2009).

These examples demonstrate the wide applicability of analyses in various types of formulation illustrating that pre-treatment of samples is not required, rapid analyses can be conducted, whilst maintaining reproducible and robust results.

I. Analysis of supramolecular complexes using IMS-MS

The syntheses of supramolecular complexes that possess photo-optical properties are desired for solar energy capture and conversion, molecular machines, photochemical drugs and fluorescent-based sensors.

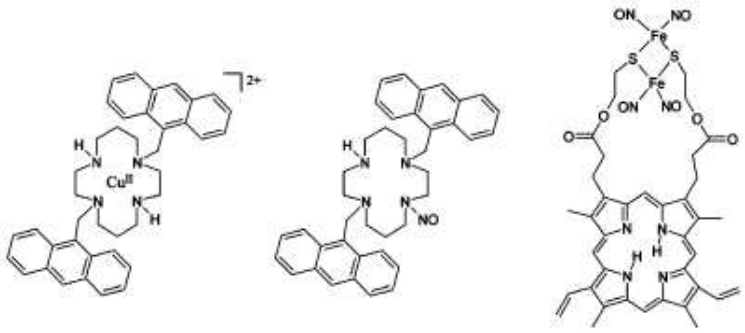

FIGURE 25. Schematic of $Cu^{2+}(DAC)^{2+}$ (**I**), DAC-NO (**II**), and PPIX-RSE (**III**), adapted from Baker et al., (2005).

DT-IMS-MS was used to probe the structures of bichromic complexes (Figure 25; Baker et al., 2005) in order to provide relevant data for sampling from *in situ* fluid data. Complementary data to ^{1}H-NMR, x-ray crystallography and fluorescence measurements were obtained.

For **I** the crystal structure agreed well with DFT structures and IMS-MS measurements, indicating that solid-state structures agreed well with gas-phase measurements. Only a single peak was observed in the ion mobilogram and calculation gave 161 $Å^2$ as the CCS, compared to 166 $Å^2 \pm 5$ $Å^2$ predicted from the DFT structure.

For **II** DT-IMS-MS measurements indicated two conformers by observation of two main bands in the ion mobilogram, by comparison with computational data this suggested two major families of Up-Up and Up-Down configuration, as shown in FIGURE 6. The solution NMR data for **II** also suggested two conformers but the structures could not be unambiguously determined from the data.

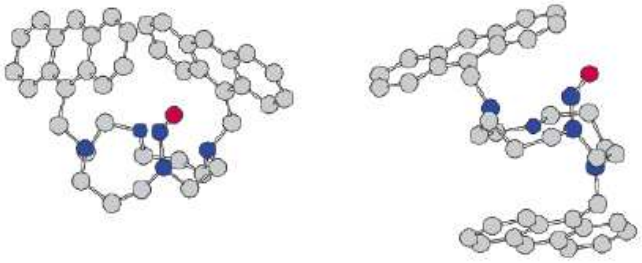

FIGURE 26. Examples of the two families predicted for (**II** + H)$^+$. The Up-Down family is the lower energy family, the Up-Up family has both anthracenyl groups on the same side as the cyclam. Adapted from Baker et al., (2005).

For **III** DT-IMS-MS measurements indicated two conformers which, in combination with DFT measurements indicated two compact structures, rather than folded structures, and correlated well with photophysical features including a bimodal fluorescent decay and a residual emission in steady-state luminescence experiments. The proportion of the two conformers measured by IMS-MS, shown in Figure 27, agreed well with pre-exponential factors that indicated an approximate 80:20 ratio.

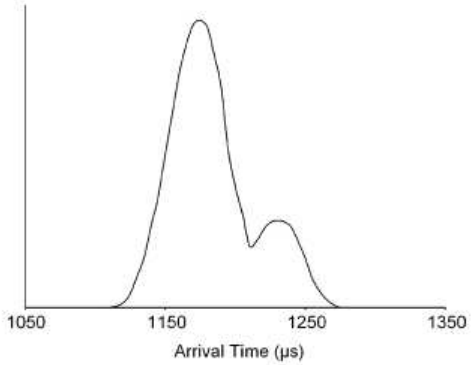

FIGURE 57. ATD for (**III** + H)$^+$ obtained at 80 K. Two distinct peaks indicate two conformers of (**III** + H)$^+$ are present, adapted from Baker et al., (2005).

J. Hydration and desolvation of ligands and substrates

In drug design it is important to consider water molecules particularly in two situations:

(i) those water molecules that will be displaced during ligand binding in a receptor (Poornima and Dean, 1995), and

(ii) those water molecules that will be desolvated crossing the membrane environment.

Water in binding pockets in a receptor can provide surprising entropic and enthalpic contributions to structure and binding affinities (Pace et al., 2004; Homans, 2007). If the key water binding sites and influence of 'small molecule' ligands are known it may be possible to use this information in medicinal drug design, or to predict static hydration sites. It may be especially important to consider bridging waters that link ligand to protein via an extended hydrogen bond network.

Understanding membrane permeability is key to drug delivery and activity and is typically understood by hydrogen bond descriptors such as polar surface area (PSA) and surrogate measurements such as logD. These are considered important physicochemical parameters and modulated during lead optimisation. The reason that these parameters are important is that it is polar groups that are most involved in desolvation when molecules move from an aqueous extracellular environment to the lipophilic membrane environment. During this migration molecules may change their conformation and lose water molecules in order to cross the membrane barrier. To further understand the effect of desolvation on ligands it is possible to add/remove water molecules one by one by changing the water vapour pressure of the DT-IMS-MS cell and gradually ascertain the ion mobility and conformation adopted from a hydrated towards a non-hydrated ion, shown in Figure 28(a). By measuring the energy change at different temperatures a van't Hoff plot can be generated, shown in Figure 28(b), thereby revealing the entropic and enthalpic contributions to hydration.

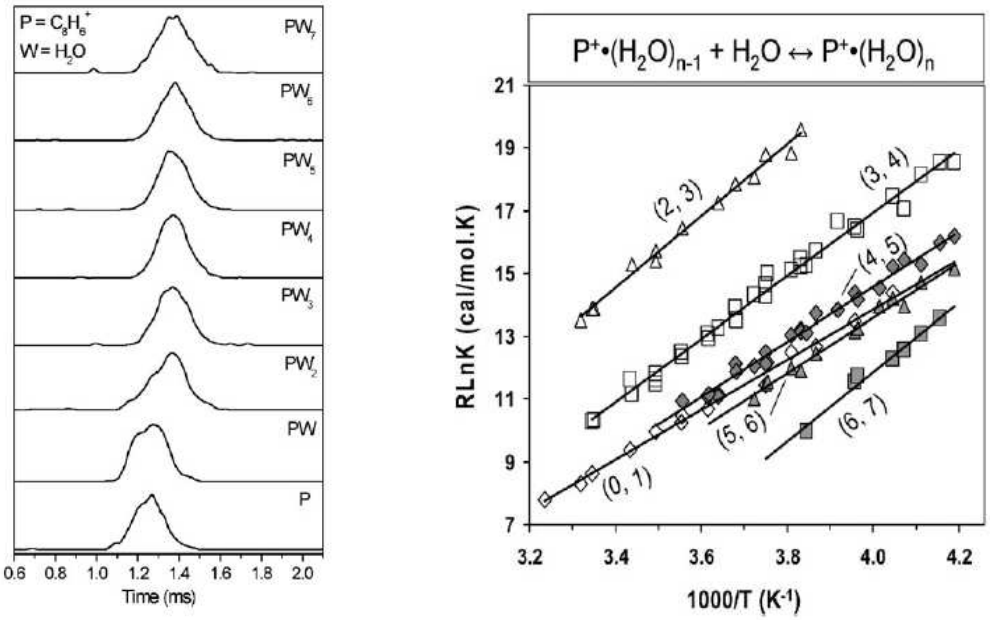

FIGURE 28. (a) ATDs of hydrated phenyl acetylene ions (PW_n) obtained following the injection of the phenyl acetylene ion ($C_8H_6^{\bullet}$+) into 0.34 Torr of water vapor at 249 K (b) Van't Hoff plots for the equilbria $C_8H_6^{\bullet}$+(H_2O)n-1 + H_2O ⇔ $C_8H_6^{\bullet}$+(H_2O)n for n-1 and n as indicated. Adapted from Momoh & El-Shall., (2008).

Hydration of small molecules has been studied for the phenyl acetylene ion, with stepwise hydration energies of 39.7 ± 6.3 kJ mol^{-1} from n=1 to 7; the entropy change for step 7 is larger, indicating a cyclic or cage like water structure (Momoh & El-Shall, 2008). For the benzene ion stepwise hydration energies were 35.6 kJ mol^{-1} from n=1 to 6. The binding energies were larger in the n=7 and 8 clusters indicating cyclic or cage like water structures (Ibrahim et al., 2005). For small protonated peptides the hydration energy is largest for highly charged peptides and small non-arginine containing peptide and typically 30 to 60 kJ mol^{-1} (Wyttenbach et al., 2003); for pentapeptides AARAA, AARAA-OMe and Ac-AARAA the binding energies were typically ~41 kJ mol^{-1}.

The foregoing IMS-MS studies indicate hydration/desolvation studies of small molecule ions can provide structural information in the gas phase, this may be relevant to:

1) understanding water and hydrogen bonded networks (including their entropic consequences) involving protein, ligand and water as part of molecular recognition systems,
2) ligand desolvation on transport through membrane environments, and
3) hydrogen/deuterium exchange experiments and how they are effected by molecular conformation.

Other methods to measure the hydration of small drug-like molecules include infra-red data recorded from a hydrated electrospray source or a droplet ion source (Poully et al., 2008) but these have not always provided unambiguous data, possibly due to the lack of energy required for proton transfer. The measurement of hydration/desolvation energies, described above, may provide a unique insight into the energy landscape of hydration/desolvation by conducting measurements over a range of temperatures.

V. OVERVIEW OF COLLISION CROSS-SECTION (CCS) MEASUREMENTS FOR SMALL MOLECULES

In DT-IMS-MS and TWIMS-MS, larger ions (with a larger CCS) tend to migrate slower through the gaseous medium in the IMS cell compared to smaller ions due to a higher number of collisions with the gas molecules (typically an inert gas such as nitrogen). The drift times through the IMS cell can also reveal structural information such as size, shape and topology; potentially including information

relating to accessible conformations. Unfortunately DMS and FAIMS are, currently, not suitable for carrying out CCS measurements.

Understanding small molecule structure in the gas-phase may be advantageous for quality control or for a more detailed understanding of molecular structure in the gas-phase. For example in drug discovery the physicochemical and binding properties of small molecules depend on their 3D structure and at physiologically relevant temperatures a conformationally flexible small molecule is expected to be able to access a number of energetically feasible conformers, an example is shown in Figure 29. The timescale of interconversion of conformers will define the structural information that can be obtained in solution and in the gas-phase. Understanding the energetics of small molecule conformers is currently largely carried out by generating potential conformers, known as conformational sampling, in computational studies (Foloppe & Chen, 2009).

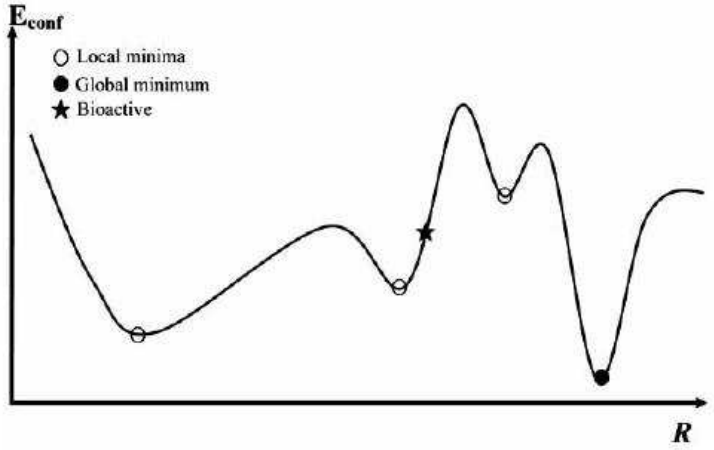

FIGURE 29. Hypothetical example of a one-dimensional molecular conformational potential energy surface. Conformational degrees of freedom (R) are shown on the X axis. Adapted from Foloppe & Chen (2009).

Computationally sampled models have been compared with x-ray crystallographic structures to understand how well the conformer models correlate with the bioactive conformation. Solution NMR can provide valuable information about the 3D structure; however the interpretation is often difficult due to the exchange between several conformations and typically requires molecular modelling to interpret results.

Rapid calculation of CCS by IMS-MS may be useful to decide which molecules in a library (series) could provide the optimum activity. This could be achieved coarsely by excluding molecules which are too rigid/flexible or too big/small as suggested by Williams et al. (2009a). These experiments potentially have the advantage of rapid speed of experiment and low consumption of sample relative to NMR and x-ray techniques. Understanding the conformation in the gas-phase may be a good indicator of the bioactive conformation. This may be especially relevant to compounds in drug discovery which are challenging to isolate and characterise their structure. Mapping the conformational landscape defined via stereo-centres, intramolecular cyclisation etc., may help uncover a path to identification of new target compounds.

For protein structures there is now significant evidence that the gas-phase protein structure can reflect the native state solution phase structure under certain carefully controlled conditions. There have been several publications that demonstrate a good correlation between x-ray, NMR and IMS studies for protein structures (Heck and van den Heuvel, 2004; Rand et al., 2009; Ruotolo et al., 2005; Schultz and Solomon, 1961; Shelimov et al., 1997; Shelimov and Jarrold, 1997), although there have also been some differences noted (Jurneczko and Barran, 2011). However for small molecular weight molecules the evidence that gas-phase structures are similar to solution phase structures has been questioned; in a protein there are multiple cooperative interactions that maintain the 3D structure whereas for a small molecule there are typically fewer interactions resulting in a more flexible structure. Furthermore Allen et al. (1996) compared a range of gas-phase and x-ray molecular substructures for small molecules and suggested that high-energy conformers were represented more in gas-phase, room-temperature Boltzmann distributions than in crystal structures and broad peaks

observed in IMS have generally been interpreted as indicating that multiple conformations are accessible and interconvert on the IMS measurement timescale.

Measurements using IMS-MS may not be the same as NMR (which are subject to solvent effects) or x-ray (which are subject to crystal lattice effects). In enzymes and membrane receptors, biomolecular recognition processes are likely to take place in hydrophobic 'binding pockets' of proteins where there will then be several interactions for a ligand including hydrophobic amino acids, with a large possibility (>0.8) of excluding most water molecules. The dielectric constant of a partial vacuum in IMS-MS (c.f. ε_{vacuum} = 1) is more similar to the immediate environment of a membrane receptor ($\varepsilon_{peptide/protein}$ = 2–4) than for water (ε_{water} = 80)(Bastug and Kuyucak, 2003). We may therefore postulate that the environment of a bioactive conformer will often be intermediate between aqueous and gas-phase (vacuum). Therefore the gas phase may be an appropriate medium in which to study the 'small molecule' structures which in their active form are bound to a receptor located in a membrane, rather than in solution.

A. CASE STUDIES OF COLLISION CROSS-SECTION (CCS) MEASUREMENTS FOR SMALL MOLECULES

1. Study of an organoruthenium complex and its adducts with a DNA oligonucelotide

TWIMS-MS has been used to understand the binding of a "piano-stool" shaped organoruthenium complex with a single stranded oligonucleotide hexamer that show promise as an anti-cancer agent. The illustration in Figure 30 shows examples of the protonated and deprotonated complexes, the doubly positive charged complex and the doubly negative charged complex. The single peak (A) suggests a single species, whereas multiple peaks in (B), (C) and (D) suggest either multiple binding of the Ru-drug fragment (confirmed by interpretation of the mass spectra collected) or different conformers present in the mononucleotide due to different charge distributions along the phosphate backbone (Williams et al., 2009a).

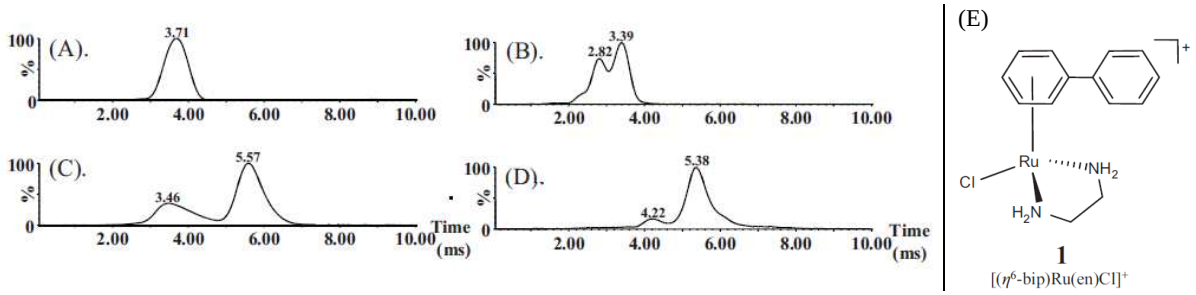

FIGURE 30. Arrival time distributions (ATDs) or drift times for (A) the $[M+2H]^{2+}$ ion of d(CACGTG); (B) the $[M-2H]^{2-}$ ion of d(CACGTG); (C) the complex $[CACGTG+2[(\eta^6\text{-bip})Ru(en)]-2H]^{2+}$; and (D) the complex $[CACGTG+2[(\eta 6\text{-bip})Ru(en)]-6H]^{2-}$ and (E) structure of the organoruthenium anticancer complex ($[(\eta^6\text{-bip})Ru(en)]$), adapted from Williams et al., (2009a).

The CCS values obtained for the Ru-based drug correlated well with those obtained by x-ray crystallographic data so that binding could be easily identified. Using MS/MS experiments, shown in Figure 31, subsequent to IMS separation, enabled the binding site to be determined by examining the resulting fragmentation pattern.

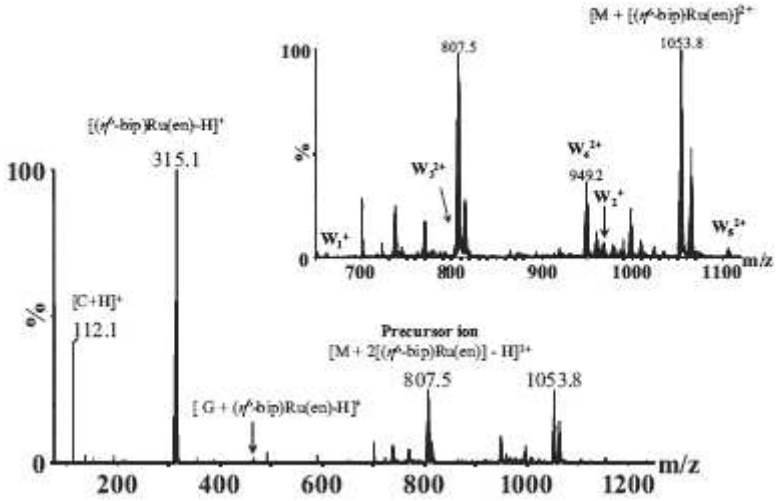

FIGURE 31. MS/MS spectrum of the precursor ion of m/z 807.5, corresponding to [CACGTG+2[(η6-bip)Ru(en)] H]$^{3+.}$ Inset shows the relevant sequence-specific ions detected. (Note: M represents CACGTG), adapted from Williams et al., (2009a).

2. Study of the in-flight epimerisation of a bis-Tröger base

The epimerisation pathway via the proposed alternatives of a) a proton catalysed ring opening or b) retro-Diels-Alder of a bis-Tröger base, shown in Figure 32, were investigated using TWIMS-MS (Révész et al., 2011) as this could be important for the design of Tröger bases which, with their tweezer type structure, have been suggested as useful agents as molecular receptors, chiral solvating agents and inclusion complexes (Maitra et al., 1995).

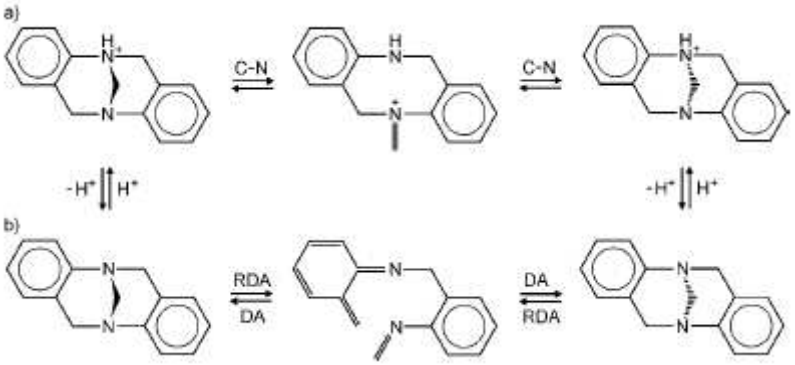

FIGURE 32. Proposed mechanism for epimerisation of a Tröger base by a) a proton catalysed ring opening or b) a retro-Diels-Alder mechanism, Révész et al., (2011).

The two structures were separated well in the gas-phase TWIMS stage (FIGURE 33) and activation of ions pre-TWIMS separation and post-TWIMS separation demonstrated that the anti-1H$^+$ isomer is the most thermodynamically favoured by measuring the intensity of each parent ion. The preferred mechanism was also concluded to be the proton catalysed ring opening as demonstrated by the lack of epimerisation when a Na$^+$ Tröger base was used as a surrogate proton-like participant in the reaction, thus eliminating the possibility of a retro-Diels-Alder mechanism.

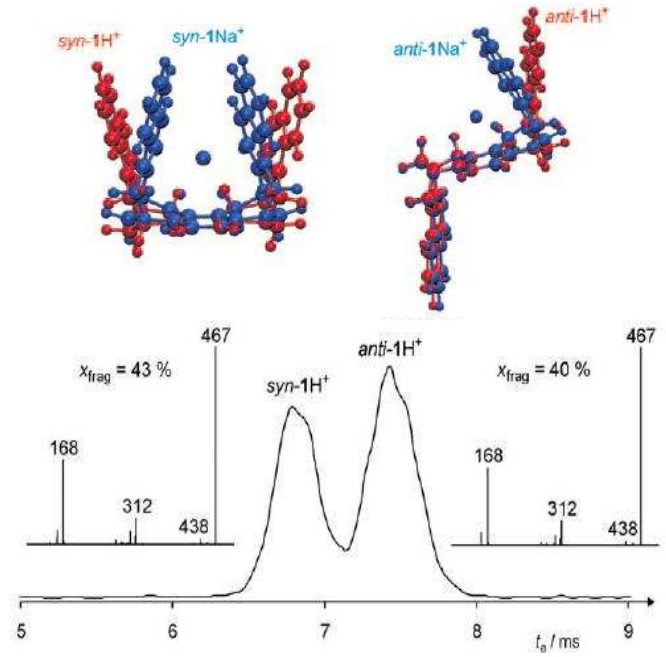

FIGURE 33. Ion mobility trace with associated mass spectra (shown inset) of the *anti-* and *syn-* isomers. The computationally calculated structures are shown above, adapted from Révész et al., (2011).

B. Measurement of collision cross-section (CCS) for small molecules using DT-IMS-MS

The measurement of CCS in DT-IMS is simplified by the use of a static, uniform, electric field in which ion motion takes place; the physical principles are established and mobility values can be used to derive the collision cross-section. Knowing the length of the drift region and the time that ions take to traverse it enables the ion's velocity to be determined:

$$v = \mathrm{KE} \rightarrow \frac{\mathrm{L}}{\mathrm{t_d}} = \mathrm{K}\frac{\mathrm{V}}{\mathrm{L}} \rightarrow \mathrm{K} = \frac{\mathrm{L}^2}{\mathrm{Vt_d}} \qquad (9)$$

where v is the ion's velocity, K is the ion mobility constant, E is the electric field, L is the length of the drift tube, t_d is the arrival time and V is the voltage across the drift region.

K should be corrected for temperature and pressure to obtain the reduced ion mobility, K_0 (corrected to 273 K and 760 Torr):

$$\mathrm{K_0} = \mathrm{K}\left[\frac{273}{\mathrm{T}}\right]\left[\frac{\mathrm{P}}{760}\right] \qquad (10)$$

The collision cross-section, Ω_T, can then be derived directly:

$$\Omega_{\mathrm{T}} = \left(\frac{3ze}{16N}\right)\left(\frac{2\pi}{\mu kT}\right)^{1/2}\left(\frac{1}{K}\right) \qquad (11)$$

where Ω is the collision cross-section, ze is the ionic charge, N is the background gas number density, μ is the reduced mass of the ion-neutral pair, k is Boltzmann's constant, T is the gas temperature and

K is the mobility constant.

C. Calculation of collision cross-section (CCS) for small molecules using TWIMS

The measurement of CCS in TWIMS is not typically directly derived from the mobility of an ion (Giles et al., 2010) as the motion of the analyte in the travelling wave regime is complicated and, to date, is not fully understood. The TWIMS system is, therefore, usually calibrated using ions that have previously been measured by employing DT-IMS. A typical calibration regime (Knapman et al., 2010) has been described based on the CCSs of oligo-glycine ions (available at http://www.indiana.edu/~clemmer/) which are currently accepted to be suitable as they are high mobility ions in the expected mobility range of small molecules, and have been measured previously using DT-IMS. Calibration with a static 4 V wave gave more drift time values over a narrower range of CCSs than a wave ramp, potentially resulting in greater resolving power. Although changes can be made to the buffer gas used in the measurement, the larger the buffer gas molecules, the larger the CCS and it was noted that the buffer gas radius used in theoretical calculations must be indicative of the buffer gas used in the original DT-IMS measurements (typically helium), even if the analysis of calibrants and analytes is carried out in a different buffer gas.

The experimental resolving power was reported under these conditions for the isomeric amino acids isoleucine and leucine (131 Da), calculated the CCS at 68.95 $Å^2$ and 70.51 $Å^2$, respectively from the measured arrival time distributions (see Figure 34).
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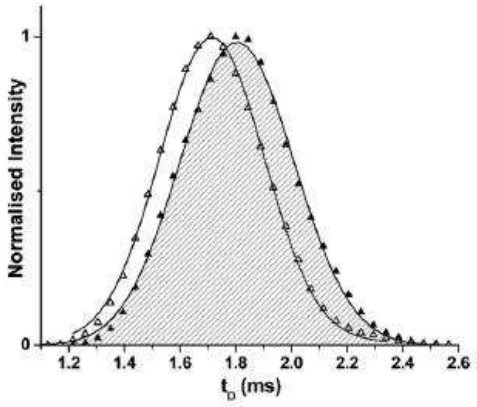

FIGURE 64. Overlaid mobility chromatograms of L-Ile (open) and L-Leu (filled) acquired using a static 4 V wave height. The ESI-TWIMS-MS experimental CCS values measured were 68.95 and 70.51$Å^2$, and the calculated CCS values were 70.81 and 72.03$Å^2$, for L-Ile and L-Leu, respectively. Adapted from Knapman et al., (2010).

To understand the differences between solution state and gas-phase measurements the theoretical collision cross-sections were calculated as a weighted average over multiple solution-phase rotameric states from a database of 5000 protein structures and compared to the experimentally measured gas-phase values (Table 4). Calculated CCS values for hydrophobic amino acids gave the best agreement with gas-phase TWIMS values, whilst more polar residues are experimentally found to be much smaller than calculated, mostly likely due to burying of polar and charged termini. The largest differences also appear to be correlated to the degrees of freedom in the amino acid side-chain.

TABLE 4. Comparison of measured and predicted CCS values for seven amino acids, adapted from Knapman et al., (2010).

Amino acid	Mw (Da)	Rotamers	TWIMS experimental CCS ($Å^2$)	CCS predicted from solution state ($Å^2$)	Difference ($Å^2$)	Notes
Pro	115.12	2	62.43	63.16	0.73	Hydrophobic
Val	117.15	3	64.81	64.82	0.01	Hydrophobic
Leu	131.12	4	70.51	72.03	1.52	Hydrophobic
Ile	131.12	4	68.95	70.81	1.86	Hydrophobic
Asp	133.11	5	62.93	69.81	6.88	Polar

Gln	146.15	5	62.85	75.08	12.23	Polar
Glu	147.13	7	63.35	76.69	13.34	Polar

This demonstrates that IMS-MS can distinguish between subtle changes in shape e.g. differentiating Leu and Ile and also has the potential to reveal structural information about the important interactions present in the gas-phase such as the burial of the polar groups in the examples Gln and Glu.

D. Calculation of collision cross-section (CCS) for small molecules using overtone IMS-MS

Recently, overtone mobility spectrometry (Kurulugama et al., 2009; Valentine et al., 2009), where separation is achieved by applying time-dependent electric fields to sequential segments in a drift-tube thus eliminating ions that are not resonant with the applied field, has been used to demonstrate measurements of ion collision cross-sections:

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_b T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_B} \right]^{1/2} \frac{E[\phi(h-1)+1]}{f(l_t + l_e)} \frac{760}{P} \frac{T}{273.2} \frac{1}{N} \quad (12)$$

where Ω is the collision cross-section, k_b is the Boltzmann constant, z is the ionic charge, e is the charge of an electron, N is the buffer gas density, T is the temperature of the buffer gas, P is the pressure of the buffer gas, E is the electric field, m_I and m_B are the masses of the ion and the buffer gas. The overtone IMS specific parameters include f which is the application frequency, h is the harmonic index, l_t is the ion transmission length and l_e is the ejection length.

Reduced ion mobilities are reported to enable a comparison of DT-IMS-MS and overtone-IMS-MS measurements and are, in general, in good agreement. An especially interesting feature is the potential for overtone-IMS-MS to exclude different ion structures i.e. with different ion mobilities in the IMS stage. Typical IMS and TWIMS approaches are thought to measure an experimental average of all structures sampled within the IMS drift time, whereas overtone-IMS-MS appears to enable selection of particular structures over the IMS drift-time, potentially giving a better understanding of transitions on the IMS measurement timescale, in the order of a few milliseconds. Current measurements are limited for small molecules but development is ongoing.

E. Using theoretical calculations to understand ion mobility data

The assignment of structural information is typically made by comparing theoretical, calculated CCS values with experimentally determined CCS values by using the following procedures:

1. generate list of conformers,
2. minimise structures to lowest energy structures,
3. calculation of theoretical CCS, and
4. comparison of theoretical CCS values with experimentally determined values.

1. Generate list of conformers

Initially the molecule must be transformed from a flat 2D to a representative 3D structure at physiological pH taking into account tautomerism, likely protonation site(s), bond lengths etc. The accessible conformations can be explored for small molecules (Dear et al., 2010; Williams et al., 2009b) using methods including systematic search, molecular dynamics, random search and grid search tools but may be very computationally expensive if the number of rotatable bonds is high, requiring evaluation of thousands of potential structures for relatively simple structures.

2. Minimise structure to lowest energy structures

Molecular dynamics approaches have been applied to small molecules with success and computationally are far less demanding than for large molecules (Baumketner et al., 2006; von Helden et al., 1995; Hoaglund-Hyzer et al., 1999; Jarrold, 2000; Kinnear et al., 2002). Methods have included force-field techniques including MMFF94 forcefield (Dear et al., 2010) and CHARM (Mao et al., 2001) but quantum mechanical methods e.g., density functional theory (DFT) may also be feasible for understanding small molecule structures. Indeed DFT has almost become the 'norm' for

calculating ion structures (Holmes et al., 1985), as it is more accurate than semi-empirical methods . Recent work (Alex et al., 2009; Wright et al., 2010) has highlighted the potential for DFT to understand electron density in bond formation/cleavage and the effect of protonation on bond lengths, which makes DFT a potentially powerful tool in modelling ion structures in IMS-MS. Indeed the information obtained from DFT calculations may contribute to a better understanding of the ion structure for both the IMS separation and any tandem MS results.

3. Calculation of theoretical CCS

The main calculation protocols for obtaining theoretical CCS values in IMS include projection approximation (PA), trajectory method (TJ) and exact hard sphere scattering (EHSS). Whilst there has been some debate about which type of modelling is most appropriate, it is generally recommended to use the projection approximation (PA) method for small molecules of 20-100 atoms, for example using the Sigma software package or MOBCAL software. However, PA typically underestimates collision cross-sections for polyatomic species, especially for different surfaces including concave structures, by up to 20% (Shvartsburg and Jarrold, 1996), so is not typically recommended for larger molecular weight structures.

TJ typically works well for any size system, but calculations are computationally expensive. Exact Hard Sphere Scattering (EHSS) typically fails with small molecules because the ion-buffer gas interaction becomes important compared to the geometry of the ion and careful calibration of the relevant atomic radii is essential (Shvartsburg and Jarrold, 1996). EHSS and TJ appear to provide better agreement for larger molecular weight ions as the parameterisation of EHSS is based on fullerenes and other large molecular weight ions.

There have been attempts to improve modelling, for large molecules (Shvartsburg et al., 2007) and small molecules (Knapman et al., 2010; Siu et al., 2010), by construction of new parameter basis sets with values for the carbon, oxygen, helium and nitrogen interaction radius calculated from suitable representative molecules. Further development of modelling and prediction techniques (Fernandez-Lima et al., 2009) and improvement in parameter basis sets may well provide closer agreement between calculated and measured CCSs. Recent improvements to a nitrogen based trajectory method (Campuzano et al. 2011) may help understand data generated in $N_{2(g)}$ (as the less polarizable $He_{(g)}$ is typically used) and create better calibrations for collision cross sections (especially useful in TWIMS where $N_{2(g)}$ is the typical drift gas). The set of collision cross sections for pharmaceutically relevant 'small molecule' compounds appears self consistent (R^2 = 0.9949) and covers a useful range of 124.5 to 254.3 $Å^2$ for nitrogen gas and a range of 63.0 to 178.8 $Å^2$ for helium gas.

4. Comparison of calculated CCS values with experimentally determined values

Typically validation is best achieved using known standards within experimental sets, either for relative ranking of results or to increase confidence in measurements. Structure co-ordination sets are widely available for some species e.g. at the RCSB Protein Data Bank and have been data based by Clemmer (available at http://www.indiana.edu/%7eclemmer/Research/cross%20section%20database/Proteins/protein_cs.htm). However, it should be noted that the co-ordination structures from different sources may not agree as NMR structures are often subject to solvent effects, x-ray structures subject to crystal lattice effects and measurements by ion mobility may be subject to gas-phase neutral contamination, ionisation and solvent effects. Some publications describe the calculation of theoretical collision-cross sections using datasets obtained from NMR and x-ray files (e.g. PDB files) as input without subsequent energy minimisation in the gas-phase which could result in erroneous estimates of CCS and further assignment; in such a case a better understanding via structure/energy minimisation may be important.

VI. PREDICTION OF ION MOBILITY CONSTANTS

Whilst many approaches to IMS explicitly use or attempt to derive information on the 3D structure of the ion another approach is to use molecular descriptors to adequately describe an ion and predict the reduced mobility without any requirement to carry out computationally expensive geometry optimisation. A quantitative structure property relationship (QSPR) methodology using five descriptors for a training set of 70 organic compounds and excluding three outliers gave a multi-linear regression (MLR) of R^2 = 0.98 and s = 0.047; the test set of seven compounds gave s = 0.047 (Wessel and Jurs, 1994). Later, using six molecular descriptors on a training set of 135 compounds and testing the model with 18 compounds gave an RMS error of 0.038 (Wessel et al., 1996). A more

diverse set of 182 compounds and modification of two of the descriptors correlated with an R^2 =0.80 (Agbonkonkon et al., 2004). A subset of 159 of that data set was used to develop linear and non-linear models using MLR and progression pursuit regression to achieve R^2 values of 0.908 and 0.938 and s = 0.066 and 0.055, respectively (Liu et al., 2007a). The recent formulation of a linear equation for ion mobility in a series of polar aliphatic organic compounds resulted in ion mobility predictions that were typically >99% accurate (Hariharan et al., 2010).

These molecular descriptor approaches are now widely used in predicting peptide IMS-MS drift times (Wang et al., 2010) to improve confidence in peptide identification. The same approach to prediction of 'small molecule' IMS-MS drift times could well help refine models of drift time prediction and better understand important interactions affecting drift time and thus gas-phase structures, however this is currently not well understood.

VII. FUTURE DEVELOPMENTS

The adoption of IMS-MS both for small molecule as well as large molecule applications is likely to continue strongly, assisted by rapid developments in IMS design that marries the two stages of IMS and MS and mitigates the challenges of ion efficiency and resolution that has hindered their combination.

The resistive glass-IMS design recently invented to replace the traditional stacked-ring ion guides enables easier construction (Kwasnik and Fernández, 2010) and designs include a segmented rf quadrupole in the vacuum interface that improve sensitivity by over 2 order of magnitude (Kaplan et al., 2010). The inverse ion mobility spectrometry technique that applies an inverted pulse to the shutter grid appears to increase resolution by 30-60% presumably by creating a gap in the charge cloud and thus reducing space-charging effects (Tabrizchi and Jazan, 2010).

A further hyphenation of a photoelectron spectrometer to a IMS-TOFMS shows promise as a complementary method to obtain further information on the structures of gas-phase ions by obtaining photoelectron spectra at three different detachment laser wavelengths (Vonderach et al., 2011), and also hints at the possibilities for further information-rich data to be acquired and combined with IMS-MS by further hypenation.

The adoption of IMS in hyphenated IMS-MS systems is continuing with important developments, for example, Agilent previously announced collaborations with Owlstone Nanotechnologies for an IMS-MS system and Bruker have investigated new modes of IMS (Baykut et al., 2009). There have been long-term research investments demonstrated in the launch of the second generation Waters Synapt G2 IMS-MS with improved resolution and ion transmission and with the AB Sciex SelexION technology that is available for the AB Sciex Triple Quad 5500 and QTRAP 5500 Systems including selection of gas-phase dopants which can improve IMS separation and rapid 25 ms cycle time per MRM which matches cycle times with multi-component analysis and UHPLC time scales.

VIII. CONCLUSIONS

Whilst IMS is a ubiquitous technique in airports as well as military and forensic applications, it is still the case that using IMS-MS for measuring structural information and for separations in 'small molecule' applications there are subtle differences that can significantly affect the mobility and there is much more to be understood about how to measure the structures of gas-phase ions reliably, the nature of the fundamental intra-molecular interactions that define the structures and what the effect of ion-neutral interactions are on ion mobility.

Many chemical classes have been investigated using IMS and IMS-MS and some of the main publications are listed in Table 5 to direct the reader to more detail on those classes.

TABLE 5. Applications of IMS-MS and IMS to 'small molecule' classes.

Class	Year
Hydrocarbons (Creaser et al., 2004)	1973
Halogenated benzenes and nitro benzenes (o-, m-substituted) (Karpas et al., 1988)	1973
Dihalogenated benzenes (o-, m-substituted) (Karpas et al., 1988)	1974

Benzoic and isophthalic and phthalic acids (Karpas et al., 1988)	1975
Some sec-butylcholorodiphenyl oxides (Karpas et al., 1988)	1976
o- and p- substituted chlorodiphenyl oxides (Karpas et al., 1988)	1976
Ethyl butyl esters of maleic and fumaric (Karpas et al., 1988)	1982
Succinic acids (Karpas et al., 1988)	1982
Isomeric ketones 2-octanone vs. 4,4-dimethyl-3-hexanone (Karpas et al., 1988)	1986
Isomeric alcohols 1-octanol vs. 2-octanol (Karpas et al., 1988)	1986
Substituted electrophilic olefins, keto enol isomers, 2 keto and 2 enol (Karpas et al., 1988)	1988
Amides and amines (Karpas et al., 1988) (Karpas et al., 1994)	1989 & 1994
Anilines (Karpas et al., 1990b)	1990
Simple monocyclic and dicyclic compounds (Karpas et al., 1990a)	1990
Aminoazoles (Karpas and Tironi, 1991)	1991
Ketones (Karpas, 1991)	1991
Aminoalcohols (Karpas, 1992)	1991
Benzodiazepines, amphetamines and opiates (Karpas et al., 1988)	2001 & 2002
Amino acids (Asbury & Hill, 2000b)	2001
Amphetamines (Matz and Hill, 2002)	2002

Standardized calibration and measurement methods (Fernández-Maestre et al., 2010b), easily implemented and accurate predictive models and interpretation of results are still being developed but show great promise. The interchange between academia, vendors and industry is ensuring more options are available to potential users of IMS-MS. A range of current commercial manufacturers and IMS-MS types are listed in Table 6 for reference.

TABLE 6. Commercially available IMS systems, or accessories able to interface to MS systems.

IMS-MS manufacturer	**Type**
Excellims IMS-MS	DT-IMS
Tofwerk IMS-MS	DT-IMS
Waters Synapt IMS-MS	TWIMS
Thermo FAIMS cylindrical electrode	FAIMS
Owlstone Nanotech	FAIMS
AB Sciex SelexION parallel plate	DMS
Sionex microDMx	DMS

Over the last decade there have been many novel applications and developments in IMS-MS involving new methods to generate ions, accumulate and focus ions, select ions preferentially, measure and process the multiplexed information and they have been used to solve problems ranging from hydration/desolvation in 'small' organic molecules to understanding the fundamental interactions in the building blocks of life, amino acids. IMS-MS is a novel method that can separate ions and use information on their mobility to assign structure on an unparalleled rapid timeframe and at high levels of sensitivity. In combination with a range of analytical equipment including ionisation sources, separation devices, solution chemistry and gas-phase chemistry; the use of IMS-MS offers a versatile and powerful approach to unique insights into complex mixtures and hitherto ambiguous structures.

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