Ion Mobility-Mass Spectrometry

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1 Introduction

Ion mobility spectrometry (IMS) separates ions based on their mobility in an inert buffer gas in the presence of an electric field. The mobility of ions is based on their size, shape, and charge, thus IMS provides insights into structure. In addition to being used for structural information, IMS can also be used as a separation device for complex mixtures. When coupled with mass spectrometry (MS), IMS–MS offers a powerful hybrid analytical technique that has many biological, pharmaceutical, structural, environmental, and other applications. This article provides an overview of IMS–MS, which focuses on principles of drift-time ion mobility spectrometry (DTIMS), high-field-asymmetric ion mobility spectrometry (FAIMS), and traveling wave ion mobility spectrometry (TWIMS) methods. Several IMS–MS instruments are discussed and examples of the current applications of the technology are provided.

1 INTRODUCTION

IMS is a powerful analytical technique that has become more widespread in the last 40–50 years. IMS has several capabilities as a stand-alone instrument and has been used to monitor the detection of atmospheric compounds, explosives, chemical warfare agents (CWAs), and petrochemical reagents. In recent years, IMS technology has been used to detect explosives and narcotics in airport scanner devices. While it has been extremely effective in field applications as a stand-alone or portable device, the coupling of IMS with MS extends the capabilities and applications of the technique tremendously. IMS–MS is extremely useful for obtaining structural information on small polyatomic ions to macromolecular ions, such as proteins and even viruses. IMS–MS instruments can be operated in modes which take advantage of IMS as a separation device allowing complex mixtures to be investigated and low-abundance species to be detected owing to the removal of chemical noise. Furthermore, IMS–MS provides fast measurements which allow it to be compatible with other front-end analytical separations, such as liquid chromatography and capillary electrophoresis.

Owing to the growing interest in IMS–MS, this article seeks to provide a general overview of IMS–MS technology. There have been several notable advances in IMS–MS instrumentation which have led to a plethora of interesting applications. The reader will be introduced to the basic principles surrounding three of the most commonly employed types of IMS separations. Current applications of IMS–MS have only been made possible due to the many advances that have taken place in instrumentation development and technology. Thus, an overview of several IMS–MS instrumentation setups is also provided. Finally, examples of several applications
2 PRINCIPLES OF ION MOBILITY SPECTROMETRY

The basic principle of ion mobility separation can be simply described as a gas-phase electrophoresis technique, whereby gaseous ions are separated according to their size, shape, and charge in the presence of a weak electric field. The drift tube is filled with an inert buffer gas. As ions move under the influence of this field, the energies of the ions are similar to the thermal energy of the buffer gas. Various ions will have different mobilities in a given drift tube device which allows the separation of mixtures of ions and structural information to be obtained. The simplest configuration of a drift tube is one in which a series of stacked ring electrodes have a static direct current (DC) field applied across the electrodes and the tube is filled with an inert buffer gas. As ions move under the influence of this weakly applied electric field, they have a velocity, \( v_D \), which is governed by the electric field, \( E \), and mobility of the ion, \( K \), in a specific buffer gas.

\[
v_D = KE
\]

\( K \) is measured experimentally based on the time it takes an ion to traverse the drift tube of length, \( L \).

\[
K = \frac{L}{t_{DE}}
\]

Comparisons of reduced ion mobilities, \( K_0 \), across laboratories can be obtained by normalizing for buffer gas pressure, \( P \), and temperature, \( T \), as follows:

\[
K_0 = \frac{L}{E \frac{273}{T} \frac{760}{P}}
\]

It is often useful to deduce information about the structure (i.e. the size and shape) of specific ions based on a mobility experiment. This is possible using an experimentally derived collision cross-section, \( \Omega \), for an ion, which represents the average area of the molecule that interacts with the buffer gas over a range of three-dimensional orientations.

\[
\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_B T)^{1/2}} \left[ \frac{1}{m_1} + \frac{1}{m_B} \right]^{1/2} \frac{t_{DE} 760}{L} \frac{T}{P} \frac{760}{N}
\]

In the above expression, \( ze \) refers to the charge on the ion, \( k_B \) is Boltzmann’s constant, \( m_1 \) and \( m_B \) are the masses of the ion and buffer gas, respectively, and \( N \) is the number density of the buffer gas. Because IMS can be coupled with MS the mass and charge of an ion can be readily deduced. By operating at specific fields (i.e. low or high) or with different pressure regimes, different IMS methods can be developed. Here we discuss three common approaches: DTIMS, FAIMS, and TWIMS.

2.1 Drift-Time Ion Mobility Spectrometry

DTIMS–MS is the most widespread developed and employed approach. DTIMS is the only IMS method which provides a direct measure of collision cross-section based on an ion’s mobility. Figure 1(a) shows a simple drift tube instrument that is filled with inert buffer gas in a counter direction of the ion motion. The weak electric field applied to the drift tube is generated using a series of resistors and a DC potential. The electric field applied is generally around 2.5–20 V cm\(^{-1}\) in reduced-pressure IMS (i.e. the drift pressure ranges from 1 to 15 mbar). Higher voltages are applied across the drift tube when higher pressures, such as atmospheric pressures, are used. Regardless of the pressure regime used, it is important that the voltages applied do not cause the potential breakdown of the buffer gas. Traditionally used buffer gases are helium, nitrogen, and argon, or mixtures thereof.

DTIMS does not work with continuous injection of ions, therefore packets of ions are introduced into the drift tube using an ion gate, or ion funnel. Ion packets can range in width from 100 to 200 \( \mu \)s. Because of the use of ion packets, the overall sensitivity of the method is reduced such that only 0.1–1% of ions generated are sent to the IMS. After the ions are injected into the drift tube, the species begin to separate based on their mobility through the buffer gas. For example, doubly-charged species experience the force of the electric field twice as much as singly-charged ions, therefore for ions of the same shape the doubly-charged ion will have a higher mobility through the tube and thus a shorter drift time. Also, ions which have more elongated conformations will undergo more collisions with buffer gas atoms and thus take a longer time to drift through the tube than more compact structures. These concepts are illustrated in Figure 1(a).

Typically, ions travel through the drift tube on the order of milliseconds which makes for a relatively fast separation. As can be inferred from the mobility equations described, the length of the drift tube can influence the transient time and mobility of an ion. The drift resolving power \( (t/\Delta t) \) at full-width half maximum...
Figure 1  Illustration of drift tube separation principles. (a) Principle of DTIMS. Packets of ions are injected into a drift tube filled with an inert buffer gas. Under the influence of a weak electric field, ions are separated by charge, size, and shape. (b) Principles of FAIMS separation. An asymmetric waveform is applied to two cylindrical plates such that ions experience alternating high and low electric fields. Ions traverse the region between the plates moving in a perpendicular direction to the buffer gas and with the influence of a DC potential, termed the compensation voltage (CV). Only selected ions at a given CV will make it through the drift region. (c) Principles of TWIMS separation. An alternating phase radio-frequency (RF) potential is applied to a series of stacked ring ion guides (SRIGs). Ions are pushed through the drift region with a traveling potential wave and become mobility separated as higher mobility ions are able to ‘roll-over’ the traveling waves generated and exit the SRIG region.

is approximated as follows:

$$\frac{t}{\Delta t} \approx \left( \frac{L E z e}{16 k_B T \ln 2} \right)^{1/2} \tag{5}$$

This theoretical resolving power approximation\(^{(29)}\) shows that increasing the length of the drift tube or applied electric fields, or decreasing the buffer gas temperature can increase the resolving power. A typical length for an in-house-built IMS drift tube is \(\sim 1\) m. Clemmer et al.\(^{(30)}\) and Bowers et al.\(^{(31)}\) have shown that increasing the tube length to 2 m or greater can significantly improve the resolving power. A circular drift tube design, which has effectively infinite length, can extend the drift resolving powers of small peptides to \(> 300.\)\(^{(32)}\) At higher electric fields, the buffer gas starts to break down, therefore higher electric fields are employed only with higher pressure drift tubes.
as mentioned earlier. Cryogenically cooled drift tubes with subambient temperatures have been recently demonstrated. The ability to work with higher resolution drift instruments allows greater separation power for complex mixtures or isomeric and isobaric species with closely related mobilities and can give better insight into structural transitions in the gas phase.

It is worth briefly mentioning, as are further discussed subsequently, that the IMS and MS separations using this approach are not completely orthogonal. Because the mass and shape of ions strongly influence ion mobility, there is a mass correlation observed in a two-dimensional (2D) IMS–MS experiment.

2.2 High-Field-Asymmetric Waveform Ion Mobility Spectrometry

At extremely high electric fields (i.e. \( > 10^4 \text{ V cm}^{-1} \)), the velocity of ions does not follow the relationship shown in Equation (1).

\[
\lambda = \frac{eE}{m_B} \left(1 + \frac{n}{2} \left(\frac{eE}{m_B} \right)^2 + \frac{3 n^2}{8} \left(\frac{eE}{m_B} \right)^4 \right)
\]

The mobility of the ions is dependent on the strength of the electric field which changes throughout the course of the experiment. As shown in Figure 1(b), there are two closely spaced (\( \sim 2 \text{ mm} \)) cylindrical or planar plates in which an asymmetric field is applied. As ions move toward each of the plates, they experience different field strengths and form an oscillating motion between the plates. For example, the field on one plate is twice the electric field that ions experience on the second plate. In contrast to DTIMS, the buffer gas is flowing in the direction of the ion motion which causes the path of the ions to be perpendicular to that of the electric field. In order for ions to traverse down the course of the electrodes and avoid hitting the electrode walls, a compensation voltage (CV) is applied to the plates. Under a given CV, only a specific ion is able to exit the end of the drift field. Therefore, a scan of increasing CVs allows a range of ions with different mobilities to be measured. FAIMS acts similar to a quadrupole mass analyzer in this way which is in contrast to DTIMS whereby all ions are transmitted simultaneously. Ion abundances are reported as a function of CVs as opposed to drift times. FAIMS also allows a continuous beam of ions to be introduced into the drift tube as opposed to a small packet of ions in DTIMS.

The exact mechanisms of FAIMS separation are not clearly understood and thus it becomes very difficult to deduce structural information from this IMS method. However, there are examples whereby FAIMS has been useful for obtaining conformations of well-studied systems. In addition, because the field strength employed is in the high-field limit the nature of the bath gas can greatly influence the ion energy and mobility.

2.3 Traveling Wave Ion Mobility Spectrometry

TWIMS has its origins in the commercialization of IMS–MS technology by Waters Corporation (see Section 3.2.1). The drift tube instrument in this case consists of a series of three stacked ring ion guides (SRIG) in which an radio frequency (RF) voltage is applied across consecutive electrodes and used to stop the radial spread of ions. Superimposed on top of the radio frequency (RF) fields is a DC voltage which is used to move ions down the axial direction of the tube. As illustrated in Figure 1(c), the DC is pulsed so that in time ions begin to ride along the wells created by the potential field. Ions continue to move forward and are separated as higher mobility ions are also able to ride over the wave. The highest mobility ions ‘roll-over’ the waves less times and have a faster transit through the SRIG. The wave amplitude, velocity and buffer gas pressure can be altered in order to optimize ion transmission through the SRIG. For specific applications, the SRIG can be used as a transmission device, storage device, or collision cell. Conformational information can be obtained from TWIMS by using careful calibrations to well-studied systems.

3 ION MOBILITY SPECTROMETRY–MASS SPECTROMETRY INSTRUMENTATION

IMS–MS began with the work performed by McDaniel et al. in the late 1950s and 1960s when he developed an IMS–MS instrument to study ion molecule reactions of noble gases and pure hydrogen. His instrument design was a low-pressure drift device that was coupled to a magnetic sector mass spectrometer. Kebarle and Hogg also created an early IMS–MS device for measuring ethylene gaseous ions. Over the last 40–50 years, there have been numerous developments in IMS–MS devices. The basic components found in any IMS–MS instrument include the source, drift tube, mass analyzer, focusing elements, and ion detector. Technological advances in each of these components have greatly added to the overall improvement of IMS–MS instruments. Different combinations of IMS and MS instruments have been realized, including IMS-time-of-flight mass spectrometers (TOF-MS), IMS-quadrupole mass spectrometers (qMS), IMS-ion trap mass spectrometers (IT-MS), IMS-Fourier transform mass spectrometers (FTMS), and IMS-magnetic sector mass spectrometers. We provide a brief overview of ionization sources and discuss the features of commonly used IMS–MS instruments in the following sections.
3.1 Sources

3.1.1 Electrospray Ionization

Electrospray ionization (ESI) is one of the most popular ion sources used in IMS–MS and MS since its discovery and application in biological molecules from the work of Nobel laureate John Fenn et al.\(^{(48)}\) ESI is a soft and continuous ionization method that is nondestructive to analytes and generates multiply-charged ions.\(^{(48)}\) The association of multiple charges on analytes extends the effective mass range of species that can be detected, thereby making molecules such as DNA and viruses accessible.\(^{(49–51)}\) In addition, other types of nonvolatile and thermally labile compounds, such as polymers and small polar molecules, are accessible with ESI.

The ESI source consists of a glass, metal, or fused-silica sample capillary which contains liquid solutions of the analyte of interest flowing at rates of 0.1–1 mL min\(^{-1}\) or higher. There is a potential drop (≈several kilovolts) that is created between the capillary and source of the instrument (in this case the drift tube, see Figure 2a). The distance between the capillary tip and the instrument source is 0.3–2 cm creating a ‘Taylor Cone’ which contains charged droplets of the analyte. As these droplets migrate toward the entrance of the instrument, the droplets shrink in size as the solvent evaporates. At a given point, the Coulombic repulsion in the droplet exceeds the surface tension and the droplets disperse into smaller droplets. This process continues until protonated analyte ions are left in the gas phase. Nebulizer and drying gases are often used in order to assist with desolvation and aid in the transfer of ions to the source. For IMS–MS instruments, such as those encountered in DTIMS and TWIMS, an ion gate is located following the ESI source.

3.1.2 Matrix-Assisted Laser Desorption/Ionization

Matrix-assisted laser desorption/ionization (MALDI) is another attractive source for the study of large molecules. MALDI is a soft ionization method and unlike ESI, it is a pulsed source and primarily generates singly-charged ions.\(^{(52,53)}\) The sample preparation procedure is simple, making it a good choice for imaging MS applications.\(^{(54)}\) In addition, MALDI has better tolerance to salts and detergents within samples. Analytes of interest are mixed with selected small organic matrix compounds (e.g. 3,5-dimethoxy-4-hydroxycinnamic acid, α-cyano-4-hydroxycinnamic acid, and 2,5-dihydroxybenzoic acid). After solvent evaporation, the matrix and analyte co-crystallize on the surface of a 96-well metal plate. The matrices have been selectively chosen so that they can absorb the radiation emitted by the laser (e.g. Nd:YAG or CO\(_2\)). Spots are bombarded with laser pulses either under vacuum source conditions, as in traditional MALDI,\(^{(52,53)}\) or at atmospheric conditions for atmosphere pressure matrix-assisted laser desorption/ionization (AP-MALDI).\(^{(55,60)}\) As shown in Figure 2(b), charged analyte and matrix ions are irradiated from the surface of the spot and are accelerated toward the IMS source. Because MALDI is a pulsed ionization technique, it works well with DTIMS as each laser shot generates a packet of ions which can be injected into the drift tube without the use of an ion gate. Several groups have employed MALDI as an ion source before IMS–MS.\(^{(21,31,57–59)}\)

3.1.3 Laserspray Ionization

Laserspray ionization (LSI) is a newly developed ionization technique that resembles AP-MALDI, however results in multiply-charged ions similar to those produced in ESI.\(^{(60,61)}\) The setup is slightly different from that of AP-MALDI, as shown in Figure 2(c). The major differences are that a transparent slide is used to house the analyte/matrix crystals, no voltage is applied to the plate to accelerate ions into the source, and the laser beam is transmitted through the bottom portion of the glass slide as opposed to directly ablating the surface of the plate.\(^{(62)}\) The distance of the sample plate to the inlet (≈1 mm) is

**Figure 2** Schematic diagram showing the mechanisms of (a) ESI, (b) MALDI, and (c) LSI.
important for minimizing sample loss. Matrices used by LSI are similar to those used with MALDI.

LSI features high sensitivity, easy sample preparation, simple laser focusing, and simple source instrumentation.\(^\text{[61]}\) The combination of LSI with IMS–MS provides a solvent-free ionization and analysis platform. Recently, Inutan and Trimpin\(^\text{[63]}\) validated this coupling with a commercial Waters Synapt G2 IMS–MS instrument on peptides and proteins ranging in molecular weight (MW) from 5.7 to 17 kDa.

3.1.4 Others

While we highlight three ionization sources which are used for a range of higher MW compounds, there are other ion sources which have been applied in IMS–MS. Early studies of small gas-phase ions and molecules also applied corona ion discharge\(^\text{[15,64]}\) and radioactive ion sources.\(^\text{[65]}\) Laser desorption ionization is useful for the ionization of solid samples and has been demonstrated on carbon clusters, silicon clusters, and fullerenes.\(^\text{[66–68]}\) Other examples of sources include ultraviolet (UV) photoionization,\(^\text{[69]}\) secondary ESI,\(^\text{[70]}\) desorption electrospray ionization (DESI),\(^\text{[71]}\) and direct analysis in real time.\(^\text{[72]}\) For more detailed information on sources compatible with IMS, we refer readers to a recent review on the subject.\(^\text{[73]}\)

3.2 Hybrid Instruments

3.2.1 Ion Mobility Spectrometry–Time-Of-Flight-Mass Spectrometry

Since the principles of TOF-MS have been proposed in the 1940s,\(^\text{[74,75]}\) DTIMS-TOF-MS has attracted considerable attention and undergone continuous development and improvement. McAfee et al.\(^\text{[19]}\) constructed the first IMS-TOF-MS in 1967 to study the mobilities and reactions of small ions in the presence of argon gas. IMS is an excellent match for TOF-MS analyzers owing to the timescales of each technique. The scan time of TOF is the order of microseconds, which is much faster than the IMS separation that occurs on the order of milliseconds. Thus a ‘nested’ IMS-TOF measurement is obtained\(^\text{[25]}\) and hundreds to thousands of MS spectra are acquired for a single IMS pulse (Figure 3a).

The basic components of an IMS-TOF-MS instrument include an ion source, a drift region, a TOF analyzer, focusing elements, and a detector. The layout of the instrument can be similar to that shown in Figure 3(b), which is a design by Baker et al.,\(^\text{[76]}\) in which the TOF analyzer is orthogonal to the drift tube. Ions generated in the ion source are injected into the drift region in packets. If a continuous ion source is used, such as ESI, an ion gate with a grid is used to pulse ion packets into the drift tube. Figure 3(a) shows an example of a typical pulsing diagram that may be used in an IMS-TOF-MS setup (see Figure 3b). A packet of ions (100–μs wide) is injected into the drift tube and mobility separated for a defined period (e.g. ∼50 ms). The mobility period is selected to correspond with the drift time of the lowest mobility species of interest. During mobility separation, TOF spectra are collected in a 50-μs window corresponding to the desired m/z range which generally spans up to 2000 m/z; although with the TOF analyzer, theoretically, there is no upper limit on the m/z to be measured. Hundreds to thousands of TOF spectra are nested within each IMS measurement.

Data generated from this experiment can be displayed in a 2D plot of flight time (or m/z) as a function of drift time, similar to that shown in Figure 4(a), for a mixture of tryptic peptides. The axes can also be switched for these plots. It can be observed from the plot that there is a mobility–mass correlation\(^\text{[77,78]}\) which demonstrates that the two techniques are not completely orthogonal. Specific trend lines appear in the spectrum, which correlate with different charge-state families, allowing for separation of multiply-charged ions generated during ESI or LSI. In cases where the TOF resolution is limited, these trend lines would allow charge-states to be assigned. Mobility–mass behavior is not limited to peptides but also occurs for proteins and can be used to distinguish different classes of compounds such as proteins, lipids, DNA, and glycans.\(^\text{[11,58]}\)

A few points should be noted regarding the IMS-TOF-MS designs. Because the drift gas pressure (e.g. 0.5–15 Torr)\(^\text{[15,16,26,31]}\) is generally much higher than the low vacuum necessary for TOF detection (i.e. 10^-6 Torr), a differential pumping ring is necessary to couple the devices. This can be done through designing an intermediate vacuum stage consisting of multipoles or other focusing lenses. Due to the differences in pressure that can occur between the ESI source and drift tube, considerable ion loss can occur because of diffusional losses.\(^\text{[46]}\) A major contribution to improve ion transmission efficiency in DTIMS-TOF-MS is the ion funnel designed and introduced by Smith et al.\(^\text{[26,80]}\) Two ion funnels are present in the instrument shown in Figure 3(b): an hourglass-shaped ion funnel after the source capillary and a second funnel located after the drift region. The design is based on a stacked ring RF ion guide, which is composed of a set of ring electrodes with opposite RF phases applied to every other electrode (Figure 3b inset). The ring electrodes have orifices with gradual decreasing diameters that focus the ions into a collimated beam at pressures up to 30 Torr.\(^\text{[76]}\) A DC gradient is also applied along the funnel to push the ions in the z-direction. The hourglass funnel is similar except for its geometry that allows...
Figure 3  (a) Example of a pulsing diagram for a DTIMS-TOF-MS experiment. The upper trace shows that an ion packet (100 μs width) is injected into the drift tube and mobility separated over the course of 50 ms. After a variable delay following ion injection, TOF scans of the order of 50 μs for a typical size m/z range, are acquired. Hundreds to thousands of TOF spectra are acquired within a single mobility experiment. (b) A schematic diagram of ESI-DTIMS-TOF-MS reprinted from Ref. 76. (Reproduced with permission from Ref. 76. Copyright 2007, Springer.) The inset shows a zoom-in of a typical ion funnel geometry.

Ions can also be collisionally activated in the IMS-TOF-MS design at regions located after the drift tube before entering the TOF analyzer. Activation is possible with skimmer cones, octopole collision cells, Triwave cells, split-fields, surface-induced dissociation, and other methods. Due to the timescale of the mobility separations and the short time of ion activation, the drift times of fragment ions are very similar to that of the precursor ions from which they arose. Figure 4(b) gives an example of fragmentation spectra for a tryptic peptide that was isolated using a quadrupole mass filter and fragmented in a collision cell. The b- and y-fragment ions detected for this peptide all have similar drift times. One advantage of
Figure 4  (a) Nested drift (flight) time distribution for an electrosprayed mixture of tryptic peptides of ubiquitin. The octopole collision cell was evacuated during the collection of these data, and the quadrupole was set to transmit all ions. The solid lines provide visual guides corresponding to the [M + H]⁺ and [M + 2H]²⁺ charge-state families. (b) Nested drift (flight) time distribution for ubiquitin tryptic mixture. The quadrupole was used to select the [TITLEVEPSDTIENVK + 2H]²⁺ ion (m/z = 849) and 3.8 × 10⁻⁴ Torr of argon was added to the octopole collision cell. Fragmentation of the [TITLEVEPSDTIENVK + 2H]²⁺ ion as well as the [TLTGK + H]⁺ ion is apparent. (Reproduced with permission from Ref. 79. Copyright 2000, American Chemical Society.)

Figure 5  Schematic of Waters Synapt G2-STM HDMS instrument. It features a Stepwave ion guide, a quadrupole, a Triwave TWIMS, and a QuanTOF MS. (Image used with permission from Waters Corp.)

IMS-TOF-MS fragmentation is that without quadrupole selection, all ions are simultaneously fragmented. This ‘parallel fragmentation’ (79,81) can increase the throughput of Tandem mass spectrometry (MS/MS) experiments.

FAIMS (41,88,89) and TWIMS (42) devices have also been coupled to TOF-MS analyzers. Waters Corporation commercialized the TWIMS-TOF-MS design in 2006 (42) in which the first instrument design was called a Synapt.
2010, the Synapt was upgraded to the Synapt G2-S HDMS (Figure 5) which features an improved performance TOF analyzer, offline transfer lens design, and updated software. The major components are an ion source (ESI or MALDI), ion guides, a quadrupole, the TriWave which includes the TWIMS, and a reflector TOF-MS. The ion beam generated is focused toward a ‘StepWave’ ion guide, in which neutral species are removed with an offline lens design. After selection or all-ion transmission in the quadrupole, ions are accumulated in a trap and transferred to an orthogonal dual-reflection TOF-MS analyzer. Although the drift resolution of TWIMS is lower than in-house-built DTIMS, the TWIMS offers high transmission efficiency and sensitivity.\(^{(42)}\)

### 3.2.2 Ion Mobility Spectrometry–Quadrupole Mass Spectrometry

Quadrupole mass analyzers can filter ions with specific \(m/z\) ratios or allow all \(m/z\) ions to pass through the detector. Although quadrupoles have slower scan speeds, lower resolutions, and mass accuracies than TOFs, they are widely used mass analyzers because of their simplicity, inexpensive cost, and utility for targeted analyses. The time required for a qMS scan is on the scale of 100 ms, which is far slower than TOF-MS and is similar to the timescales of DTIMS separations. Thus, it is more practical to use single-ion monitoring instead of full \(m/z\) scans in an IMS–qMS setup.

The hybrid IMS–qMS instrument setup was introduced in the 1970s\(^{(64)}\) for monitoring ion–molecule reactions of oxygen and nitrogen species. The instrument mainly consists of three parts (the ion source, a DTIMS, and a qMS) located in separate chambers with small orifices to sustain the pressure differences. A DTIMS–qMS instrument with improved resolution has been reported\(^{(90)}\) and is shown in Figure 6. The drift tube in this instrument is operated under ambient pressure conditions and the buffer gas can be manipulated with the addition of modifiers that help achieve chiral selectivity.\(^{(91)}\) The Excellims Corporation offers a commercial atmospheric pressure DTIMS coupled to a qMS.\(^{(92)}\) The drift tube in this configuration is 10.85 cm. AB Sciex released an IMS–qMS instrument with different IMS electrode geometries\(^{(93)}\) and a triple quadrupole/quadruple ion-trap MS.

Wytttenbach et al.\(^{(15)}\) implemented a funnel between the ESI source and reduced-pressure drift tube to focus the ion beam and prevent high-energy ion-neutral collisions. More recently, a DTIMS has been coupled to a triple qMS for explosive detection.\(^{(94)}\) The triple qMS offers additional scanning modes and greater sensitivity than a single quadrupole analyzer. Cylindrical electrode FAIMS designs, which offer higher sensitivity than flat plate electrodes, have also been coupled to qMS.\(^{(95)}\) Because FAIMS can act as a filter to eliminate chemical contaminants, the signal-to-noise (S/N) ratio can be improved.\(^{(96,97)}\)

### 3.2.3 Ion Mobility Spectrometry–Ion Trap Mass Spectrometry

IMS-IT-MS with quadrupole ion traps (QITs, also known as Paul ion trap) and linear ion traps (LITs) have been reported.\(^{(18,98,99)}\) The coupling of DTIMS-IT-MS requires an ion gate at the IT-MS entrance to select a defined drift-time window. Clowers and Hill\(^{(18)}\) have implemented a dual-gate design. ESI-generated ions are pulsed into the

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**Figure 6** A simplified drawing of DTIMS–qMS instrument. A heated desolvation region was designed to help in desolvating the solvent. (Reproduced with permission from Ref. 91. Copyright 2006, American Chemical Society.)
drift region by the first ion gate, separated in the drift region, selected by the second ion gate, focused through the ion guides, and mass scanned by the QIT. Recently, Zucker et al. interfaced a DTIMS to a Thermo LTQ Velos instrument which features a dual LIT design. Unique to this design is the introduction of a UV laser at the back end of the second LIT for UV photodissociation experiments. In this instrument, both UV photodissociation and collision-induced dissociation (CID) can be employed to fragment specific m/z ions that are isolated in the LIT.

FAIMS coupled to a modified LTQ has been reported and commercialized by Thermo Fisher. Thermo Fisher provides the FAIMS interface in its triple quadrupole instrument (i.e. TSQ Quantum) and with LTQ and Orbitrap analyzers. The FAIMS is in a heated cube electrode design, in which selected ions can travel through a gap between two electrodes and enter the transfer capillary of the MS. This technology offers the advantage of reduced chemical noise from ESI or atmospheric pressure chemical ionization (APCI) sources and complex samples before MS analysis.

### 3.2.4 Ion Mobility Spectrometry–Fourier Transform Mass Spectrometry

The timescales of FT ion cyclotron resonance (ICR) MS do not make it the most attractive choice of mass analyzer for IMS coupling as scan times can be on the order of seconds. However, because of the inherent high mass resolving powers attainable with FTICR-MS, a number of IMS applications can benefit from this hybrid setup. Coupling IMS to FTMS is similar to IMS-IT MS in which ion gates before and after the drift tube are required. Figure 7 shows an IMS-FTICR MS constructed by Tang et al. which is composed of a nano-ESI ion source, a DTIMS, an interface region containing ion transfer optics, and an ICR cell. The interface between the IMS and ICR cell serves as an intermediate pressure region before the high vacuum required in the ICR cell (i.e. 10^-10 Torr). Although the duty cycle (more than 99% of the ion signal is wasted) has a long way to be improved for this setup, there are sophisticated dynamic multiplexing approaches to combat these issues as has been recently presented on Orbitrap FTMS instruments. FAIMS-FTICR-MS analysis on polymers has shown that this setup offers the advantages of higher sensitivity, extended dynamic range, high mass resolution and accuracy, and lower limits of detection due to the elimination of chemical noise with FAIMS.

### 4 MULTIDIMENSIONAL ION MOBILITY SPECTROMETRY–MASS SPECTROMETRY

For the analysis of simple systems, DTIMS, TWIMS, or FAIMS may provide enough increased peak capacity and separation space to identify all components in combination with MS. However, for many applications (as discussed subsequently) of biological nature, the complexity of the system still requires increased dimensionality in order to detect more components, detect low-abundance species, resolve isomeric and isobaric compounds, and extend dynamic range. To this end, IMS–MS can be connected with other front-end separations.

#### 4.1 Liquid Chromatography-Ion Mobility Spectrometry-Mass Spectrometry

Liquid Chromatography (LC)-IMS-MS has been previously reported and is one of the most straightforward front-end separations to couple with IMS–MS.
This is primarily because of the timescale of the separation and the generation of ions using sources such as ESI. In comparison to IMS, liquid LC separations occur on the order of hours with typical peak widths ranging from 15 to 45 s. This relatively long separation allows the IMS–MS measurement to be introduced at no additional cost in experiment time. Essentially, a three-dimensional ‘nested’ experiment is performed particularly if the IMS-TOF-MS platform is used. This multidimensional separation method can dramatically increase the analytical peak capacity and reduce the chemical noise to benefit the analysis of complex samples, such as proteomes\(^{114}\) or metabolomes\(^{110}\).

The inclusion of MS/MS is often necessary to confidently identify biomolecules\(^{111,115,116}\). LC-IMS-MS/MS has been demonstrated in several applications of peptides and proteins\(^{115,117}\) and generates a four- to five-dimensional data set which is gigabits to terabits in size. Sowell et al.\(^{111}\) used nanoflow LC-DTIMS-MS/MS to separate mixtures of intact small MW proteins, demonstrating the effectiveness of this multidimensional approach to characterize the overall shapes of proteins and collisionally activate precursor ions on the timescale of an LC separation (Figure 8).

### 4.2 Capillary Electrophoresis-Ion Mobility Spectrometry-Mass Spectrometry

Capillary electrophoresis (CE) was coupled to FAIMS-MS for the study of complex lipopolysaccharides\(^{118}\). They used a microspray interface to connect the CE eluent to the front end of a FAIMS-triple qMS. Due the limited number of reports using CE with IMS–MS, there is opportunity to make this coupling more desirable.

### 4.3 Ion Mobility Spectrometry-Ion Mobility Spectrometry-Mass Spectrometry

A more straightforward approach to increase the dimensionality and peak capacity of IMS–MS technology is to exploit the mobility separation space. During the past decade, ion mobility spectrometry-ion mobility...
Figure 9  Schematic of ESI-FAIMS-DTIMS-qTOF-MS instrumentation. (Reproduced with permission from Ref. 119. Copyright 2005, American Chemical Society.)

Figure 10  (a) Schematic of DTIMS-DTIMS-TOF-MS instrument. (Reprinted with permission from Analytical Chemistry.) (b) Multidimensional DTIMS-MS spectra. DTIMS-DTIMS drift-time distributions of electrosprayed bradykinin ions shown on two timescales: the total drift time (bottom) and the drift time observed after mobility selection (tD2), that is shifted by the selection time (as indicated by the dashed vertical line). Part (a) shows the distribution for all ions and includes an inset to show the low-intensity feature that is often associated with [Mn + nH]n+ ions. Part (b) shows the feature-labeled C after it has been selected at G2. Part (c) shows the distribution of fragment ions that are observed when C is activated at IA2 using 126 V. (Reproduced with permission from Ref. 16. Copyright 2006, American Chemical Society.)

spectrometry-mass spectrometry (IMS-IMS-MS) and FAIMS-IMS-MS instruments have been reported. Tang et al.\(^{(119)}\) reported a FAIMS-IMS-TOF MS instrument which is shown in Figure 9. Ions produced from the ESI emitter are filtered by the FAIMS, focused, and pulsed into the DTIMS. The advantage of this approach is that the separation principles of FAIMS and IMS are different, thus this coupling is completely orthogonal.

Koeniger et al.\(^{(16)}\) also constructed a two-stage IMS-IMS-MS instrument using the DTIMS technology (Figure 10). Ion funnels connect the two drift tube sections which are \(\sim 1 \text{ m}\) in length and activation grids...
are applied at each funnel to allow manipulation of fields which can conformationally or collisionally activate ions. The total drift resolving power of this instrument is improved to \( R = 80–160 \).\(^{(16)}\) One mode of operating this instrument is to select a small window of ions from the first IMS separation by applying a gate (G2) at the second funnel which only allows specific mobility-selected ions to traverse to the second drift region. This mobility-selected window can be transmitted, activated, or fragmented before separation in the second IMS region. Figure 10(b) provides example data from such an experiment performed on bradykinin ions. The spectrum on the bottom shows the drift distribution for the doubly- and triply-charged ions in which three and two conformer peaks are detected, respectively. A second experiment was initiated in which only the C conformer of the triply-charged bradykinin peak was isolated and detected. CID was carried out on this isolated C conformer in the second funnel and the fragments were allowed to mobility separate in the second drift region (D2), and are shown in the top spectrum.

Other examples of IMS-IMS-MS instruments include a recently reported DTIMS-FAIMS-qITMS hybrid instrument\(^{(120)}\) and a 2D overtone mobility spectrometer (OMS) by Clemmer et al.\(^{(121)}\) We refer the reader to recent reports of OMS which explain details of this IMS separation method.\(^{(121–124)}\)

4.4 Ion Mobility Spectrometry-Ion Mobility Spectrometry-Ion Mobility Spectrometry-Mass Spectrometry

To date, the only IMS-IMS-IMS-MS instrument has been reported by Merenbloom et al.\(^{(30)}\) It is similar in principle to the DTIMS-DTIMS-MS discussed above; however, it provides more stages of separation and activation. Multidimensional IMS selection and activation makes it possible to analyze more complex mixtures and extract low-intensity features in the samples. Furthermore, this multiple mobility dimensionality is important for studies that seek to characterize the nature of gas-phase conformations of proteins.\(^{(125)}\) Such insight would not be attainable without the high resolving powers and selection capabilities offered by IMS-IMS-MS or IMS-IMS-IMS-MS instruments.

5 APPLICATIONS OF ION MOBILITY SPECTROMETRY–MASS SPECTROMETRY

IMS–MS has been applied to a broad range of applications because of its ability to provide increased separation space, removal of chemical noise, and structural insight into molecules. Here we only provide a few examples as highlighted in Figure 11 to give the reader an insight into the advantages that can be gained.
from using the combined IMS–MS approach. There are other reviews that may be helpful for obtaining an insight into additional applications of IMS–MS.\(^{(3,126)}\)

### 5.1 Proteomics

The complexity of mixtures encountered in proteomics is enormous, such that multidimensional separations before MS are necessary to obtain depth in proteome coverage. It is clear that IMS has the capability to detect low-abundance species which otherwise go undetected in an MS spectrum (see Figure 4a). As mentioned earlier, the incorporation of IMS–MS into a chromatographic separation occurs at no experimental cost, therefore thousands of additional species can be detected and/or identified with high throughput. In addition, the drift times of peptides can be used along with the chromatographic retention time to ‘map’ peptides in multidimensional space.\(^{(127)}\) Peptide mobilities have specific signatures which can be correlated with chemical characteristics such as hydrophobicity.\(^{(128)}\) Several groups have used IMS–MS for bottom-up proteomics analysis of biological samples such as human plasma,\(^{(104,120)}\) mouse plasma,\(^{(112)}\) \textit{Drosophila},\(^{(114,127,130)}\) and other standard tryptic peptide libraries,\(^{(131–133)}\) combinatorial peptide libraries,\(^{(133)}\) and other standard tryptic peptide mixtures.\(^{(78,104,119,134)}\) Sowell et al.\(^{(111)}\) reported on the use of LC-IMS-MS for top-down proteomics analysis of intact proteins; however, this is an area that could be further explored. Biomarkers of disease, such as cancer, are also being explored using IMS–MS with MALDI imaging.\(^{(135,136)}\)

### 5.2 Probing Structural Information

Early applications of IMS–MS probed structures of small MW species, such as polyatomic ions and small inorganic clusters,\(^{(117–139)}\) fullerenes,\(^{(140)}\) and atomic cations,\(^{(141)}\) With the advent of ESI and MALDI, gas-phase conformations of higher MW species, such as proteins,\(^{(142–145)}\) DNA,\(^{(50,146,147)}\) RNA,\(^{(148)}\) and even viruses,\(^{(149–151)}\) have been accessible with IMS–MS. Characterization of gas-phase structures has relevance for understanding biological pathways in diseases, such as Alzheimer’s disease.\(^{(152–155)}\) Since the commercialization of TWIMS, a number of groups have used IMS–MS to study biophysical parameters of proteins and protein complexes.\(^{(12,13)}\) These complexes have higher order tertiary and quaternary structures and dynamic binding events which are only partially understood with traditional biological and other analytical techniques, such as nuclear magnetic resonance and X-ray crystallography. IMS–MS applications of protein complexes have led to a better understanding of oligomeric tryptophan RNA binding attenuation protein complexes.\(^{(148)}\) Hepatitis B virus capsids which are 3–4 MDa in size,\(^{(149)}\) and GroEL chaperone systems in \textit{Escherichia coli},\(^{(156)}\) to name a few. Much of the understanding of small inorganic systems to large biological systems with IMS–MS is made possible through computer modeling algorithms which give insight into shapes and sizes of molecules measured with experimental collision cross-sections.\(^{(12,13)}\)

### 5.3 Lipidomics

Lipidomics research, which studies the pathways and networks of cellular lipids, is currently a rapidly growing area in IMS–MS.\(^{(157)}\) IMS–MS offers high-throughput analysis, class identification, and structural information of complex lipid samples, such as cellular membranes and phospholipids. One of the earliest studies of lipids with IMS–MS reported on the ability of MALDI-IMS-TOF MS to distinguish sphingomyelin from peptides and oligonucleotides ionized from the same mixture.\(^{(58)}\) The sphingomyelin ions had lower mobilities than the peptide and oligonucleotides and thus had a distinctive trend line in the 2D spectrum. Since these studies, other reports investigating various classes of lipids were published.\(^{(157)}\) Generally, the separation of lipids in IMS–MS is due to differences in the acyl chain length, degree of unsaturation, nature of the polar head group, and cationization of individual species.\(^{(157)}\) Different classes of phospholipids in complex tissue extracts and slices have been studied with IMS–MS.\(^{(89,158)}\) The analysis of unsaturated phosphatidylcholines (PCs) with TWIMS-TOF MS identified a high mobility–mass correlation for saturated PC cations which was less significant for unsaturated PC cations.\(^{(159)}\) Trimpin et al.\(^{(160)}\) demonstrated the strengths of multidimensional DTIMS-MS for reducing complexity of lipid samples, detecting low-abundance lipids, and elucidating structure in a global lipidomics analysis of phospholipids.

### 5.4 Metabolomics

Metabolomics studies focus primarily on the analysis of metabolites for the purpose of early disease detection and prevention.\(^{(161)}\) Chemical properties of metabolites, such as polarity, solubility, and volatility, can vary tremendously among different classes. IMS–MS has the capability of analyzing complex and diverse samples with high accuracy, high peak capacity, and fast speed and has been applied in target metabolomics analysis. For example, opiates and primary metabolites were analyzed by DTIMS-TOF MS such that metabolic isomers were separated based on mobility.\(^{(162)}\) False-positive detection of methamphetamine in hair by stand-alone MS was improved with DTIMS–qMS analyses.\(^{(163)}\) Furthermore, the usage of IMS–MS has extended to metabolite...
profiling. With IMS–MS, more than 200 intracellular E. coli metabolites containing lipids, inorganic ions, volatile alcohols and ketones, amino and nonamino organic acids, hydrophilic carbohydrates, etc. were tentatively assigned.\(^{(164)}\) Quantitative IMS–MS studies have been used to monitor metabolic changes in rat lymph fluid caused by dietary stresses.\(^{(165)}\) Key to this work was the use of positive- and negative-mode ionization for the detection of amino acids, sugars, triglycerides and diglycerides in the presence of fatty acids and sugars, respectively. Pharmaceutical metabolite profiling of levunomide and acetaminophen has been reported\(^{(166)}\) and suggests that IMS–MS is suitable for routine metabolomics analyses.

### 5.5 Chiral Species

In some instances, typical IMS–MS separations are able to provide enough resolution so that isomeric and isobaric species are resolved.\(^{(167–169)}\) However, in other cases there are generally two IMS–MS strategies which are used to obtain enantiomeric separation.\(^{(91)}\) The first is to add a selected chiral reference compound to the solution that contains analyte enantiomers. Diastereomeric complexes are formed by the chiral reference compound and analyte enantiomers, which can be physically separated in the drift tube. For example, separation of D- and L-lactic acids was possible with FAIMS-MS by spiking the analyte mixture solution with excess L-tryptophan.\(^{(88)}\) Also, six pairs of amino acid and terbutaline enantiomers were characterized with IMS–MS through conversion of the enantiomers to metal-bound trimeric complexes of the form \([\text{M}^{+} 2\text{L-Ref})\text{(D/L-A)-H}]^{+}\).\(^{(117,170)}\) ESI is the preferred ionization method with this chiral approach so that the interactions between the enantiomers and chiral reference are not disrupted.

The second strategy of chiral separations, as mentioned earlier, is to dope the drift gas with a chiral modifier. For example, (S)- and (R)-atenolol enantiomers were separated with DTIMS-MS after the N\(_2\) drift gas was doped with (S)-(+) 2-butanol.\(^{(91)}\) There are other reports of IMS–MS being employed for chiral separation of small molecules including amino acids, pharmaceuticals, and sugar enantiomers.\(^{(171)}\) The reader is referred to a recent review of IMS–MS for chiral analyses.\(^{(172)}\)

### 5.6 Chemical Warfare Agents

IMS is considered the most efficient technique for the detection of CWA for its high speed, simplicity, low cost, and portability.\(^{(4,7)}\) However, as a stand-alone technique it suffers from low resolving power, limited identification ability, existence of false-positive results, and sample matrix effects.\(^{(8)}\) Most of these disadvantages can be overcome when IMS is combined with MS.

Hill et al.\(^{(17,87,173–176)}\) used DTIMS-qMS to test degradation products of common nerve agents and sulfur mustard gas in liquid samples. Different experimental conditions were investigated to optimize the separation efficiency and sensitivity of CWAs’ detection including the ionization method (ESI, SESI, CI and Ni\(^{(63)}\) radioactive ionization), ionization mode (positive/negative), drift voltage, temperature, and matrices.\(^{(117,87,175–176)}\) ACPI FAIMS-MS has been applied to detect nanogram per milliliter levels of CWAs in food and water samples.\(^{(177)}\) DESI with TWIMS-MS has also been applied in the detection of common organophosphorus CWAs.\(^{(178)}\) While the number of reports in this area is limited, it is clear that the use of ambient ionization methods is critical for extending CWA analysis to portable IMS–MS devices in the future.

### 5.7 Pharmaceuticals

IMS–MS is growing in use in pharmaceutical detection for its fast speed, low detection limit, and high accuracy. However, it is unlikely that IMS–MS will be used for online quality control in pharmaceutical production processes owing to the relatively high costs of MS, however, it is a powerful technique for in-lab characterization and direct formulation analysis of pharmaceuticals.\(^{(9)}\)

Improvements in the accuracy of amine drug detection are possible with IMS–MS over LC-MS techniques because of the ability of IMS–MS to remove metabolite interferences.\(^{(116)}\) The successful quantification of amphetamine, methamphetamine, and methylenedioxy derivatives in human urine matrix demonstrated the power of IMS–MS in the separation of rather complex pharmaceutical samples.\(^{(179)}\) A strategy which can facilitate the detection of active pharmaceutical ingredients in complex matrices has also been reported by using polyethylene-based ‘shift reagents’ in IMS–MS analysis. These shift reagents can form noncovalent complexes with the active pharmaceutical ingredients which move them to different regions of the 2D mobility–mass space.\(^{(180)}\) IMS–MS has been applied for the analysis of benzodiazepine drugs\(^{(181,182)}\) and a range of over-the-counter and prescription tablets and cream formulations.\(^{(183)}\)

### 5.8 Environmental

There are a very limited number of reports of IMS–MS use for environmental analysis. For example, haloacetic acids were quantified from complex solutions with FAIMS-MS to obtain nanogram per milliliter detection limits for samples without preconcentration or derivatization.\(^{(184)}\) A class of important triazine pollutants were analyzed with FAIMS-MS.\(^{(185)}\) Clearly, this

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is an area which can be further explored with IMS–MS technology.

6 CONCLUSIONS AND FUTURE OUTLOOK

IMS–MS represents a powerful coupling of analytical technologies as demonstrated based on the numerous applications and advantages discussed in this review. There is still room for instrumental improvements to advance the technology further and make it more accessible to analytical, biological, industrial, and even clinical laboratories. The availability of more commercial IMS–MS instruments will assist in this regard. The potential of IMS–MS for proteomics, metabolomics, lipidomics, pharmaceutical, and other applications is of great interest and should be explored with this hybrid approach.

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ABBREVIATIONS AND ACRONYMS

2D Two-Dimensional
AP-MALDI Atmosphere Pressure Matrix-Assisted Laser Desorption/Ionization
APCI Atmospheric Pressure Chemical Ionization
CE Capillary Electrophoresis
CID Collision-Induced Dissociation
CV Compensation Voltage
CWAs Chemical Warfare Agents
DC Direct Current
DESI Desorption Electrospray Ionization
DTIMS Drift-Time Ion Mobility Spectrometry
ESI Electrospray Ionization
FAIMS High-Field-Asymmetric Ion Mobility Spectrometry
FTMS Fourier Transform Mass Spectrometers
ICR Ion Cyclotron Resonance
IMS-IMS-IMS-IMS-MS Ion Mobility Spectrometry-Ion Mobility Spectrometry-Ion Mobility Spectrometry-Mass Spectrometry
IMS-IMS-MS Ion Mobility Spectrometry-Ion Mobility Spectrometry-Mass Spectrometry
IMS Ion Mobility Spectrometry
IT-MS Ion Trap Mass Spectrometers
LC Liquid Chromatography
LITs Linear Ion Traps
LSI Laserspray Ionization
MALDI Matrix-Assisted Laser Desorption/Ionization
MS/MS Tandem Mass Spectrometry
MS Mass Spectrometry
MW Molecular Weight
OMS Overtone Mobility Spectrometer
PCs Phosphatidylethanolamines
QITs Quadrupole Ion Traps
qMS Quadrupole Mass Spectrometers
RF Radio Frequency
SESI Second Electrospray Ionization
S/N Signal-to-Noise
SRIGs Stacked Ring Ion Guides
TOF-MS Time-Of-Flight Mass Spectrometers
TWIMS Traveling Wave Ion Mobility Spectrometry
UV Ultraviolet

FURTHER READING


REFERENCES

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