9 Gas-Phase Ion Mobility-Mass Spectrometry (IM-MS) and Tandem IM-MS/MS Strategies for Metabolism Studies and Metabolomics

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9.1 INTRODUCTION

Metabolite analysis is a demanding initiative for analytical technologies. It is estimated that between 4000 and 20,000 distinct metabolites are expressed in eukaryotic organisms, spanning between seven and nine orders of magnitude in concentration range [1,2], which necessitates highly sensitive and broad dynamic range analyses. This is particularly true when considering xenobiotic metabolites, in which all feasible biotransformations that an administered drug may undergo must also be considered. These may occur over a large dynamic range of possible concentrations, and depending on the dose administered, may require highly sensitive analytical techniques [3]. Metabolism is a dynamic process, requiring measurement strategies that provide high sample throughput and instrument duty cycle. A further challenge is that metabolites represent a diverse set of chemical classes possessing disparate polarities, such that there is no single extraction or separation technique that can handle most, much less all, metabolites present. Because of the shortcoming of current technologies, contemporary profiling strategies are necessarily focused on a relatively small number of known metabolites and thus are noncomprehensive. Nevertheless, targeted analysis and

Encyclopedia of Drug Metabolism and Interactions, 6-Volume Set, First Edition.
Edited by Alexander V. Lyubimov.

low complexity metabolite profiling approaches have been highly successful in drug discovery and development [4,5].

Established bioanalytical methods for studying metabolites are predominately based on nuclear magnetic resonance (NMR) and mass spectrometry (MS). MS methods are widely used and are routinely combined with additional separations [e.g., gas chromatography (GC) and liquid chromatography (LC) before MS identification] to improve instrument sensitivity and dynamic range to detect lower abundance analytes. In parallel with advances in MS has been the development of postionization gas-phase separations based on ion mobility (IM) [6–8]. IM is a compartmentalized technology that offers a combined separation and analysis that is ca. four to six orders of magnitude faster than GC and LC, respectively [9]. Since gas-phase ionization is a prerequisite for both IM and MS, the two methods are commonly coupled into a single analysis technique referred to as *IM-MS*, which allows separations on the basis of two physical properties: IM (size) and ion mass (weight). IM-MS is still an emerging technology, and one major challenge associated with using IM-MS for metabolite profiling is the limited amount of validation that can be drawn from the literature. Another challenge is the limited orthogonality between IM and MS separation dimensions, since analyte size and mass are two closely correlated physical properties [10]. Nevertheless, the extremely high speeds of the separation (micro- to milliseconds) and the potential for enhancing existing MS methodologies offer great promise for IM-MS to become a complimentary bioanalytical technique for dynamic metabolic and metabolomics analyses.

This chapter provides an overview of the IM technique and a review of the various analytical approaches that utilize IM for metabolomics. The coupling of IM and MS is discussed in light of the analytical advantages and the additional information gained in these two-dimensional analyses. In recent years, high resolution mass spectrometers have allowed for the determination of metabolite identity based on high mass accuracy measurements, which allow for the determination of metabolic transformations based on mass defect filtering, which is further enhanced in combination with IM. Tandem MS experiments (MS/MS) are important methods for elucidating analyte identity, and the common methods of operation are outlined. The addition of IM capabilities to MS instrumentation permits several useful combinations of tandem experiments (e.g., IM-MS/MS, MS/IM-MS, and MS-IM-MS/MS), which enhance the current commercial MS/MS capabilities; these are discussed in detail at the end of this chapter.

9.2 AN OVERVIEW OF ION MOBILITY TECHNIQUES

IM can be broadly defined as the *kinetic behavior* (velocity) of ions in a neutral gas. Differences in the velocity of two ions, even those isomeric in mass, can be used to differentiate the two ions experimentally. The analytical technique that utilizes the property of IM to separate ions is often called *IM spectrometry* to draw parallels with MS. Other names such as gas-phase electrophoresis [11] and plasma chromatography [12] have also been used. In terms of the technology, IM spectrometry closely resembles MS; however, the separation mechanism is very similar to chromatography, which can cause confusion if the fundamentals of the IM method are described from either (a spectrometric or chromatographic) perspective [13]. In this chapter, we refer to the analytical technique simply as IM and make important distinctions where necessary.

From a pedagogical perspective, all IM techniques can be conceptualized as a series of opposing vector forces: (i) the electric field force that directs ions across a defined region and (ii) the opposing force of ion–gas collisions that impedes this forward motion. In the simplest IM experiment, ions are pulled by an electric field through a chamber filled with a constant pressure of gas and disperse in time based on differences in their mobility. The number of ion–gas collisions is related to the size of the ion. Thus, for two ions of equal mass but different sizes, the smaller of the two will have a higher ionic mobility (velocity) and traverse the region the fastest, arriving on the other side of the region first. This kind of IM experiment is known as the *drift tube IM technique* and is the most commonly encountered IM method in the literature.

The term *ion mobility* does not refer to any single experimental method but it encompasses a family of different analytical techniques, all of which utilize the same basic concept of an ion's gas-phase mobility to generate an analytically useful separation. As with MS, IM techniques can be broadly divided into two categories: (i) temporally dispersive methods, which separate all ions in time but along the same spatial path, and (ii) spatially dispersive methods, which separate ions along different trajectories. The conceptual differences between temporal and spatial dispersion are depicted in Fig. 9.1. Temporally dispersive IM methods can obtain a complete IM spectrum in a single measurement cycle, while spatially dispersive IM techniques while spatially dispersive IM techniques transmit only a narrow IM range transmit only a narrow IM range of ions at a time. Members of the spatially dispersive IM category are considered scanning methods, which filter ions based on their mobilities and require that a parameter (typically a voltage) be scanned in order to generate the broadband spectrum. Table 9.1 contains a listing of several different IM methods along with their defining attributes. The distinction between any two IM techniques can be made by comparing the relative direction of the various field forces (electric field and gas flow) with respect to the motion of the ion. Note here that the ion motion will preferentially follow one of the forces such that the ion velocity vector is aligned with one of the force vectors. A listing of the analogous MS method for each IM technique is also provided in Table 9.1.

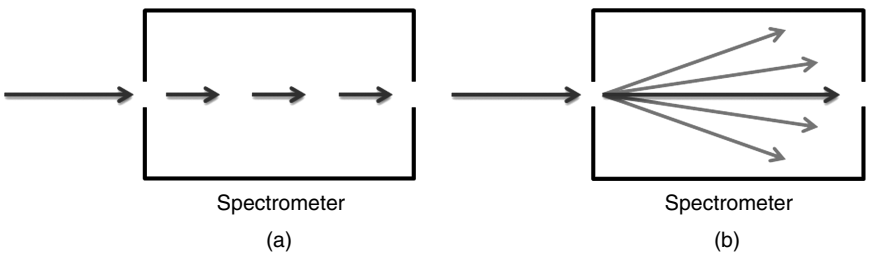

Figure 9.1 Conceptual schematic of the two types of separation schemes for ion-mobility techniques. (a) Temporally dispersive separation methods partition a pulse of ions into different mobility values along the same spatial trajectory. Ions of different mobilities arrive at different times during each measurement pulse. (b) Spatially dispersive separation methods partition a continuous beam of ions into different mobility values along different spatial trajectories, with each trajectory representing ions possessing common gas-phase mobilities.

TABLE 9.1 The Different Ion Mobility Techniques, Their Commercial Availability, Defining Features, and Currently Achieved Resolving Powers Reported in the Literature, Including the Analogous Mass Spectrometry Methods for Each Ion Mobility Technique

	Ion Mobility Technique	Commercial IM-MS Instruments (Available as of 2010)	Vectors			Resolving Power[a] Achieved	MS Analog	References
			Ion Motion	Electric Field	Gas Flow			
Temporally dispersive	Drift tube ion mobility Ion mobility spectrometry Plasma chromatography Ion chromatography	ESI-IM-Q (Excellims); www.excellims.com MALDI-IM-TOF (Ionwerks); www.ionwerks.com ESI-IM-TOF (Tofwerk AG); www.tofwerk.com	→	→	0	240[b]	Time of flight	7,9
	Traveling wave IM	Synapt HDMS (Waters); www.waters.com	→	→→→	0	40[c]	"Solitron" (mass selective traveling wave[d])	14
Spatially dispersive (scanning)	Differential high/low field IM High field asymmetric waveform IMS (FAIMS) Differential mobility spectrometry (DMS)	FAIMS (Thermo Scientific)[e]; www.thermo.com DMS interface (Sionex)[e]; www.sionex.com	→	→	↑	200[f]	Quadrupole	15,16

Differential mobility analyzer (DMA) Gas-phase electrophoretic molecular mass analyzer (GEMMA)	DMA interface (SEADM)[e]; www.seadm.com Aerosol TOFMS (TSI); www.tsi.com	→	↑	→	50[g] to 80[h]	Magnetic sector	17,18

References are provided directing the reader to comprehensive reviews on the separation described.

[a] It should be noted that resolving power does not adequately represent the separation efficiency of certain ion mobility methods, particularly the spatially dispersive IM techniques. For these methods, peak capacity is a more representative metric of separation ability (Section 9.3.2).

[b] A resolving power of >300 has been observed for a special circular path drift tube that operates in a narrow bandpass mode 19,20.

[c] Experimentally observed on the Synapt Generation 2 (G2) for data obtained from the authors' laboratory.

[d] Ref. 21.

[e] Offered as a front-end IM interface for a variety of commercial MS instruments.

[f] Ref. 22.

[g] Ref. 23.

[h] Performance specifications for the SEADM DMA interface.

The following sections describe each of the specific IM techniques in more detail. We start with a qualitative description of the drift tube IM method and use concepts discussed there as the foundation for understanding other IM techniques. These methods are initially discussed in the absence of MS; however, the IM techniques are framed as modular rather than as stand-alone or end detection technologies. Governing equations, although important for each technique, are avoided in this chapter in order to convey the qualitative operational principles clearly. The reader is directed toward specific references given in Table 9.1 if a more thorough understanding of each technique is sought.

9.2.1 Drift Tube Ion Mobility Spectrometry

The drift tube IM technique is the most familiar IM method and has been a commercial technology since the 1970s, although it has primarily been used as a stand-alone technology for chemical screening in security applications [24]. Drift tube instruments can operate at either ambient pressure (ca. 760 Torr) or reduced pressure (ca. 1–10 Torr), the latter more amendable to coupling with vacuum MS techniques. Ambient pressure drift tubes do not need bulky vacuum systems, and owing to the high gas number densities, the IM separation occurs in a relatively short distance, so these instruments are often preferred as low cost, portable chemical detectors. Reduced-pressure drift tubes are more costly and technically complex due to the added vacuum hardware, but they offer very high measurement precision and accuracy and are readily coupled to mass spectrometers. Complicating ion chemistry is often avoided at reduced pressures, where ions experience fewer interactions with trace gas impurities inherently present in the drift gases used. Reduced-pressure drift tubes are generally preferred in research applications where high quality ion measurements are desired, but one major disadvantage has traditionally been that these instruments were not readily available commercially. This situation has changed in the last five years or so as several commercial vendors have begun offering high performance drift tube IM-MS equipment.

The basic components and operational principle of the drift tube IM technique are depicted in Fig. 9.2a. A uniform electric field is defined along the length of a tube by a series of stacked ring electrodes. The tube is filled with an inert gas, usually helium or nitrogen, which is maintained at a constant pressure and low flow conditions. A pulse of ions is introduced at one end and allowed to drift down the length of the tube by the influence of the electric field via electrophoretic migration. During the ion's drift, it collides many times with the drift gas ($>10^4$ collisions at 1 Torr), which slows the ion's forward motion. The amount of time any given ion spends in the drift tube is directly related to the number of collisions it experiences, which in turn depends on its collision cross section or nominal gas-phase size and shape. This is analogous in principle to GC, where the less volatile (lower vapor pressure) analyte will spend more time in the column. The measured drift times in the IM experiment are essentially the same in principle as retention times measured in chromatography, but it is important to note that IM is not chromatography in the strictest sense, as there is no stationary phase (and thus no phase partitioning) but a constantly moving density of gas.

Because the electric field, gas pressure (number density), temperature, and tube length are (ideally) all kept constant in the drift tube IM experiment, the relationship between the measured ion drift time and the collision cross section is direct and can

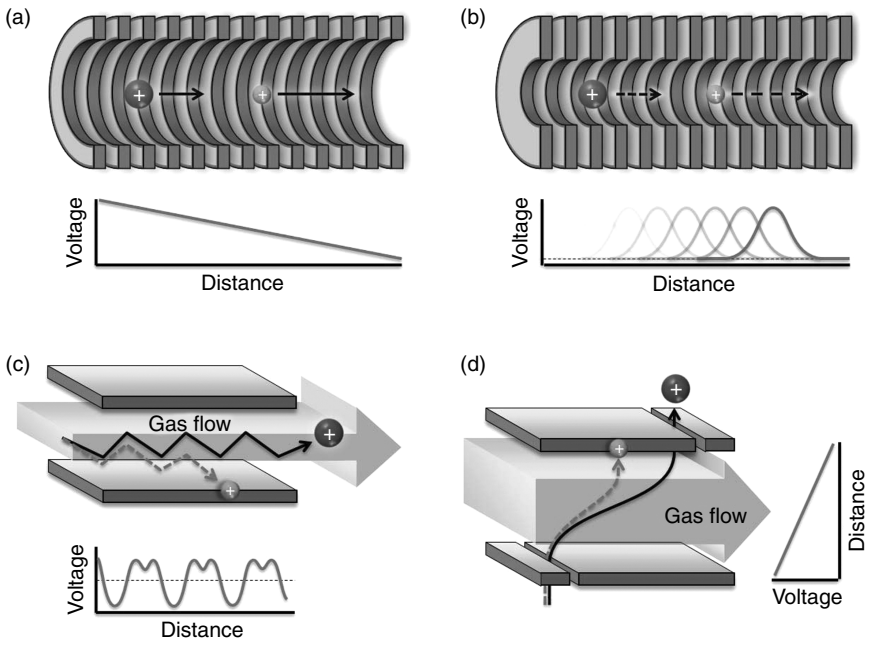

Figure 9.2 The operation principles governing the four ion mobility techniques. (a) The drift tube ion-mobility method disperses ions of differing mobilities temporally along the same trajectory by utilizing a continuous declining potential across a gas-filled region. (b) The traveling wave ion-mobility method achieves temporally dispersive mobility separations using a traveling wave potential across a gas-filled region. (c) The high field asymmetric waveform ion-mobility method disperses ions spatially along different trajectories using an orthogonally applied asymmetric waveform as ions are entrained in a gas flow. (d) The differential mobility analyzer disperses ions spatially by mobility using a flow of gas and an orthogonally applied uniform electric field.

be determined from first principle kinetic equations [25]. The measurement of absolute gas-phase collision cross sections of ions is an important feature of the drift tube experiment. The interpretation of the collision cross section value is, however, not straightforward since effects such as neutral gas polarization can contribute to nonideal (inelastic) ion–gas interactions [26]. This is particularly significant for small molecules such as metabolites, where the analyte polarity can contribute to a more attractive ion–gas interaction (a "sticky" collision), which will make the collision cross section appear larger than the physical size of the gas-phase analyte ion. Thus, it is difficult to assign any physical meaning to the collision cross sections measured for small molecules in the IM experiment. For larger analyte ions, greater than about m/z 500, the size effects become the significant contributor to the measured drift times, and in this case, collision cross section is a close representation of the physical size and shape of the gas-phase ion. The use of nonpolar drift gases also improves the accuracy of the collision cross section measurement, which is why helium is often preferred in research drift tube instruments. One prevailing advantage of drift tube instruments over the other IM techniques described in this chapter is the very high resolving powers that

can be achieved (>200) [19], which makes drift tubes important IM instrumentation for core research and development.

9.2.2 Traveling Wave Ion Mobility Spectrometry

Traveling wave is a relatively new IM technique but has already shown great promise to become a routine method for chemical analysis. This is largely due to the rapid commercialization of the technology, which is now widely available integrated into a high end mass spectrometer platform [14]. The first commercial traveling wave instrument was released in 2006 (Synapt HDMS, Waters) and has since seen a major revision (Synapt G2, 2009), which has improved the IM resolving power by about a factor of 4 (currently ca. 40–50) and offers mass resolving powers of up to ca. 50,000. The traveling wave concept itself can be traced back to early accelerator physics applications [27] and early work with using radio frequency (RF) fields to determine ionic mobilities [28,29], but until recently, there have been no attempts made at translating this technology to separating ions by mobility on an analytical scale.

The basic components of the traveling wave IM device is shown in Fig. 9.2b and are essentially the same as what can be found in a drift tube IM instrument. The drift region consists of a stacked ring ion guide and is filled with a low static pressure (1–5 Torr) of nitrogen. The rings serve two purposes: (i) a sinusoidal RF potential is applied to confine ions to the center axis of the device to improve instrument sensitivity, while (ii) a pulsed voltage is applied on sets of rings and rapidly switched from one end of the device to the other to generate the traveling wave potential for IM separation. Ions that are introduced to the device in narrow pulses will be picked up by the wave and pushed down the length of the drift region where they experience many collisions with the nitrogen gas. Some ions experience more collisions with the drift gas and are pushed against the wave until they fall behind it and wait for the next wave to carry them forward again. The number of times any given ion will tumble behind the wave is directly related to its ionic mobility—larger ions are inherently more difficult to push forward than small ions. Thus, the device disperses ions based on their mobilities in much the same way as the drift tube experiment, although fundamentally this is achieved in a very different manner. Because the wave travels at a fixed velocity, only ions that fall behind the wave will separate—those ions that are pushed along by a single wave will arrive at the same drift time and result in no useful mobility separation. Factors such as the wave velocity and voltage amplitude must be tuned in order to obtain a good mobility separation. The current second-generation traveling wave instrument (Synapt G2) can achieve IM resolving powers of ca. 45 (authors' laboratory).

Because of the complex potentials utilized in the traveling wave IM instrument, there is no direct first principle correlation between measured drift times and collision cross sections. For those interested in obtaining structural information from the IM experiment, this is seen as a disadvantage when using the traveling wave IM technique, though, methods have been proposed. By preparing a calibration curve with standards of known collision cross section, structural values have been extracted using traveling wave IM. This method is limited to the range of the calibration curve, which typically limits this method to the analysis of a particular biomolecular class. Additionally, methods of dynamically changing the traveling wave velocity for the determination

of mobility have been presented as well, though, this method is not commercially available and not particularly feasible for complex sample analysis. However, there are two distinct advantages that traveling wave IM offer over traditional drift tube IM. First, the axially confining electric fields result in very little ion loss and thus very good analyte sensitivity. Second, the traveling wave device is much easier to couple to other instrumentation due to the fact that the potential at the start and exit of the device remains the same. In contrast, coupling instrumentation such as MS to a drift tube IM device requires either the front or back component be biased to a nonzero electric potential, increasing technical complexity. A third advantage might be that traveling wave instruments are becoming much more accessible to mainstream analytical research and will no doubt see some exciting applications in the near future, particularly in the area of drug research and discovery.

Both the drift tube and traveling wave IM techniques disperse ions temporally along the same space, which permits an entire broadband mobility spectrum to be generated in each experimental measurement cycle. This has the obvious advantage of allowing one to obtain data on several analyte peaks at the same time. One limitation of temporally dispersive approaches is that ions are introduced in pulses, requiring that each measurement be complete before another pulse is admitted (a duty cycle), which reduces the overall sample throughput of the technique. In some cases, it is desirable to only monitor a few select analyte signals continuously, and so the next two IM techniques discussed act as narrow bandpass mobility filters and are particularly well suited for analyte monitoring applications.

9.2.3 High Field Asymmetric Waveform Ion Mobility and Differential Mobility Spectrometry

Consider now a continuous stream of ions entrained in a gas flow that passes between two parallel plates (Fig. 9.2c). One plate is kept at a constant potential, usually ground, and on the other plate is applied an alternating (AC) voltage. This AC voltage is kept at the same average potential as the opposite plate but alternates between a short duration, high amplitude and a longer duration, low amplitude (an asymmetric waveform) (Fig. 9.2c). The total magnitude of each push/pull potential is the same, such that the ions experience the same average electric field for each cycle, but in opposite directions. If the mobility of the ion during the push cycle is the same as during the pull cycle, the ion's average displacement will be zero. However, the gas-phase mobility of an ion has an electric field dependence such that it changes between going from high field (ca. 20,000 V/cm) to low field (ca. 1000 V/cm) [30]. This results in a different net displacement of the ion when subjected to the short duration, high field wave cycle than when it experiences the longer duration, lower field wave cycle. Ultimately, this results in ions that are not balanced by the asymmetric waveform to be neutralized onto one of the plates. This device will transmit a narrow bandpass of ions whose high field and low field IM is exactly matched by the waveform. An additional compensation voltage is applied to either plate to allow the device to be scanned, thus filtering ions possessing different changes in high/low field ion mobilities.

Devices that utilize this principle of IM separation have been described by several names, the two most popular being high field asymmetric waveform ion-mobility spectrometry (FAIMS) and differential mobility spectrometry (DMS). In Table 9.1, we refer to both techniques as *differential high/low field IM* . Both terms describe the same

general technique with defining differences being in the geometry of the electrodes used and how ions are detected in stand-alone applications. FAIMS and DMS devices do not separate purely by IM but by the difference in high/low field IM (the first derivative), so the observed separations are often subtly different from those derived from the other IM methods. In many cases, these devices can resolve ion species that are difficult to separate by traditional IM methods, such as leucine and isoleucine [31]. Additionally, one can couple FAIMS or DMS with a temporally dispersive IM method (i.e., drift tube or traveling wave IM spectrometry) and achieve excellent orthogonality between the two separations [32], which is analogous in principle to two-dimensional GC using polar and nonpolar columns. FAIMS and DMS have been successfully used to separate a variety of small molecules and both technologies are commercially available as front-end separators for mass spectrometers (FAIMS, Thermo Scientific, and DMS, Sionex). Because these devices operate at or near ambient pressure, they are readily compatible with atmospheric pressure ionization sources.

The high/low field IM behavior and transition between differential behaviors are still poorly understood. Because there is no first principle correlation between the change in IM and the low field collision cross section, analyte size information is difficult to obtain with any accuracy using the differential high–low field IM technique. Gas-phase collision cross sections can be determined in differential high–low field IM through special energy loss experiments using triple quadrupole MS [33].

9.2.4 Differential Mobility Analyzer

Differential mobility analyzers (DMAs) are a special class of IM instruments used primarily for measuring the size of aerosol particles >5 nm in diameter. These instruments are not commonly used for small molecules due to high diffusive ion losses; however, the method is included here to complete the discussion of IM technologies. The reader may skip to the next section if more relevant discussion regarding IM techniques for metabolite analysis is preferred.

Building on the differential high/low field IM technique described in the previous section, consider now a similar experimental setup, whereby ions are entrained in a flowing gas and allowed to pass between two parallel plates as shown in Fig. 9.2d. Instead of an alternating voltage, a direct voltage is applied across the two plates creating a uniform electric potential gradient perpendicular to the ion transit. This will cause ions to migrate from one plate to the other as they are being pulled along by the gas flow. All ions of the same charge will experience the same force of the electric field, but because of differences in their ion mobilities, smaller ions will be displaced at a shorter distance than larger ions. This results in a spatial dispersion of ions based on their ionic mobilities. Practical designs of this type of transverse field IM instrument utilize small apertures or slits on the parallel plates, which are offset at some defined distance from one another, as depicted in Fig. 9.2d. Ions are introduced to the gas stream through one slit, and the voltage is tuned to allow ions with different mobilities to pass through the second slit, in much the same way as tuning the m/z of a magnetic sector MS instrument. With the exit slit design, the instrument operates in a scanning mode. The exit slit can also be replaced by an array detector, which allows for broadband IM spectrum to be measured [34]; however, this design cannot be coupled as a front end to MS (e.g., IM-MS).

These instruments are sometimes called *aspirator mobility analyzers* due to the aspiration effect of introducing ions into the gas stream. More commonly they are referred to as *differential mobility analyzer (DMA)* and by the trade name gas-phase electrophoretic molecular mobility analyzer (GEMMA), the latter now referred to as *macro*IMS (TSI Incorporated). The DMA nomenclature is oftentimes confused with the DMS technique described in the previous section, but it should be noted that these two IM techniques separate ions by two very distinct mechanisms, specifically the DMA differentiates ions based on aerodynamic ionic mobility (IM in the presence of a gas flow), whereas DMS exploits IM differences between high and low electric field conditions. DMA and GEMMA have been highly successful in measuring particle sizes down to the nanometer range [17]. Smaller ions are more difficult to transmit due to diffusive losses and the instrument performance is inherently tied to the size of the slits used—larger slits improve sensitivity at a cost of resolution and vice versa. Recent technological improvements show promise for extending the usable size range down to small molecular ions with high sensitivity and IM resolution [23].

As with drift tube IM instruments, DMA measurements can be used to determine the ion's effective size since there is a direct relationship between the experimental conditions (transmission voltage and gas flow rate) and the experimentally measured gas-phase IM. A hard sphere model is used to relate the measured IM value to an effective size. As mentioned previously, DMA instruments are currently limited to the analysis of relatively large ions (tens of nanometers or greater in diameter), requiring that effects such as surface roughness be accounted for in the size calculation [35]. Nonetheless, because the DMA experimental separation parameters (voltage, drift length, and gas flow) are well characterized, the resulting measurement precision of the DMA technique is high.

9.3 INTEGRATED ION MOBILITY–MASS SPECTROMETRY

All the IM methods described previously can operate as a stand-alone chemical analysis technology (i.e., an IM spectrometer). One shortcoming of IM analysis alone is that the measured ionic mobility is a relative property of the analyte and will change with respect to different experimental conditions (e.g., drift gas pressure, temperature, and composition). Consequently, confident analyte identification using IM spectrometry information alone (via the reduced mobility) relies on rigorous method validation and even then is subject to specific constraints on the system being studied [36–38]. In contrast, MS measures an inherent physical property of the analyte (mass or more specifically, the mass-to-charge ratio), which can be used for analyte identification purposes, as the property of ion mass is readily comparable across instrument platforms. MS and tandem MS methods are the cornerstone technology of many analyte characterization initiatives, a fact that is reflected in the numerous contributions to this volume. Owing to the requirement that the analyte be ionized, IM is particularly suited for coupling with MS. The first IM-MS instruments appeared in the 1960s in response to the need to better characterize the ion chemistry observed in drift tubes [39,40]. Bioanalytical drift tube mass spectrometers emerged in the 1990s owing to the development of soft ionization methods (MALDI and ESI (electrospray ionization), which are discussed in other chapters) [6]. IM-MS is now widely utilized and regarded as a technique in and of itself [41], much like the integration of LC to MS (LC-MS). Most

of the technical challenges associated with coupling the elevated pressure IM stage to a vacuum MS have been solved, largely due to parallel initiatives in improving the transfer efficiency of ions from atmospheric ionization sources to MS [42]. As mentioned previously, commercially available IM-MS instruments are now emerging and offer little to no compromise in performance as compared with the familiar MS platforms in which they are coupled. Thus, researchers familiar with existing commercial MS technologies will find that IM capabilities enhance existing methodologies built upon MS analysis.

9.3.1 Ion Mobility–Mass Spectrometry Instrumentation

Figure 9.3a illustrates the basic components of an IM-MS instrument. Sample introduction can include direct infusion, GC, or LC. Virtually every ionization source that has been used in MS has been coupled to an IM-MS instrument [41], including all the ionization techniques covered in this volume. Likewise, virtually every combination of IM and MS has been attempted. Temporally dispersive IM (drift tube and traveling wave) combined with temporally dispersive MS (time of flight, TOF) is a particularly useful combination since both techniques offer broadband analysis for each experimental cycle [43]. Another useful combination is coupling a scanning IM (e.g., FAIMS or DMS) to a scanning MS (an ion trap), which allows selective monitoring and analysis experiments [44]. Instruments based on bracketing an IM between two mass spectrometer stages (MS-IM-MS) have also been developed [45], allowing several useful stages of tandem ion experiment to be conducted [46]. We discuss various combinations of tandem IM/MS in the later sections. Commonality between all these instrument configurations is the back-end mass analysis, which provides a comprehensive characterization of the sample by mass or, specifically, the mass-to-charge ratio. For the purposes of this chapter, we focus primarily on IM-MS instruments that incorporate the first stage of mass selection and tandem capabilities with a high resolution mass spectrometer (the so-called high performance IM-MS, Fig. 9.3b). Although the literature defines high resolution mass spectrometers with a degree of disparity, we consider high

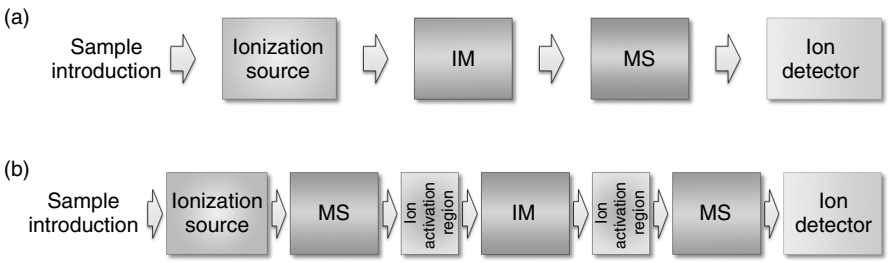

Figure 9.3 (a) A schematic of the basic components of an ion mobility–mass spectrometer. From left to right: sample is introduced, ionized, then separated first by ion mobility and then by mass, and finally, detected. (b) A "high performance" ion mobility–mass spectrometer can include a mass spectrometer before the ion-mobility spectrometer and several stages of ion activation located between each spectrometer component. Additionally, the spectrometers operate with high precision (resolving power) and sensitivity, enabling high quality measurements of mobility and mass to be made.

resolution as the ability to obtain part-per-million mass precision (e.g., 1000±0.015 Da, or 20,000 resolving power) within the mass-to-charge range of interest.

9.3.2 Orthogonality and Peak Capacity in Ion Mobility–Mass Spectrometry Analyses

The observed mobility–mass correlation in IM-MS data limits the orthogonality of the two separations. Completely orthogonal measurements would distribute data throughout two-dimensional separation space with no correlation between the data sets, as each separation is contingent upon chemical or physical properties that are independent of one another [47]. In other words, data projected on a two-dimensional separation plot would occupy all the separations space, as illustrated conceptually in Fig. 9.4a, where analyte signals distribute themselves across the full two-dimensional area. This high orthogonality is often observed, for example, in 2D gel electrophoresis [48,49], where the two dimensions of separation are analyte mass and isoelectric point—two unrelated analyte parameters. The degree of orthogonality of IM-MS separations is low due to the correlation of mass and size [50]. This is apparent when considering an ion of increasing mass, which will inherently possess a larger volume and therefore occupy an established region of separations space, a mobility–mass correlation termed *conformational space* [51]. Low orthogonality inherently limits the peak capacity (ϕ) of the IM separation, which is the maximum number of resolvable peaks within the separation window, as illustrated in Fig. 9.4b for a hypothetical IM-MS spectrum [52]. Here, the

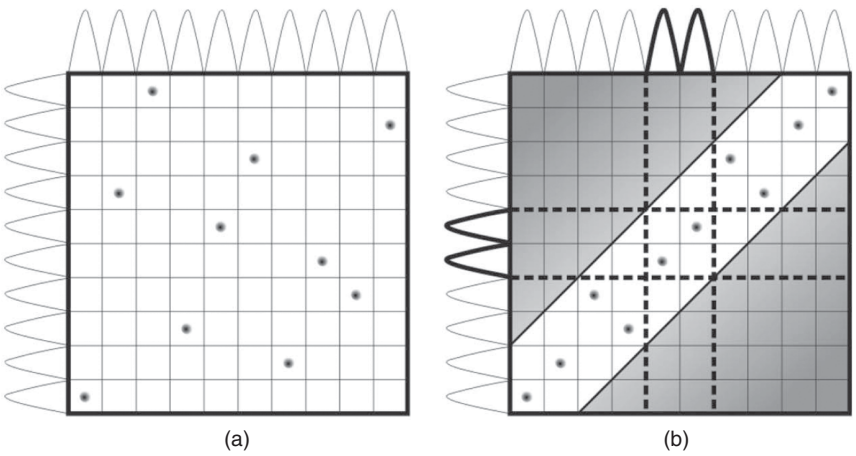

Figure 9.4 (a) A theoretical distribution of peaks (represented in black in grid space) in two-dimensional space for completely orthogonal separations. Each axis corresponds to a theoretical separation parameter. The high orthogonality allows for the occupancy of all two-dimensional space, thus having a peak capacity equivalent to the product of the two peak capacities. (b) Two separations with low orthogonality. The region of two-dimensional separations space that peaks occupy is limited to a fraction of all separations space. The region of analysis space is narrowed to that shown in the overlapping regions, for example. This limits peak capacity, as the total peak capacity is no longer the product of the two peak capacities, but a corrected value that is a fraction of the maximum. The gray areas are regions of separation space, in which no signal appears.

narrow distribution of data requires that the analysis window be narrowed accordingly, depicted with the dotted lines in Fig. 9.4b. Previous IM studies have reported a peak capacity of ~5 for the IM dimension alone, which is low when compared with other separation methods [10]. However, the strength of IM is that when combined with MS, the overall peak capacity of the MS dimension is increased multiplicatively (specifically, fivefold, for this example). As there is virtually no compromise in analytical performance when IM is added to MS, this represents a significant improvement in analytical performance. For example, IM-MS has recently demonstrated a peak capacity of 10^3 for metabolites in a single IM-MS spectrum, which is comparable to that of highly orthogonal methods, such as reverse phase LC-MS ($\phi = 1000$–1500, *Shewanella oneidensis* lysate) [53], two-dimensional GC-MS (GC × GC-MS, $\phi = 1200$, lean C57BL/6 mice spleen extract, $\phi = 1800$ human serum) [54,55], and capillary electrophoresis-MS (CE-MS, $\phi = 1692$, *Bacillus subtilis* extract) [56]. These examples represent general separation benchmarks for each analytical platform, and while useful for qualitative comparative purposes, it should be noted that the samples, conditions, and mass spectrometers are not consistent across each study.

9.3.3 Ion Mobility–Mass Spectrometry Analysis Speed

The main strength IM holds over conventional separations techniques is that it occurs post ionization, which means separations are performed entirely in the gas phase. This results in a separation that occurs on the order of hundreds of microseconds to milliseconds. As a result, thousands of IM spectra can be acquired each second. When compared with previously presented separation methods that are utilized before ionization (e.g., LC, CE, and GC), the time required for a mobility separation to occur is several orders of magnitude faster. An analysis speed comparison of several separation techniques is provided in Fig. 9.5. For a typical metabolomics study, high performance LC, two-dimensional GC, and CE separations occur on the order of minutes to hours. Additionally, GC separations often require analyte derivatization to promote volatility,

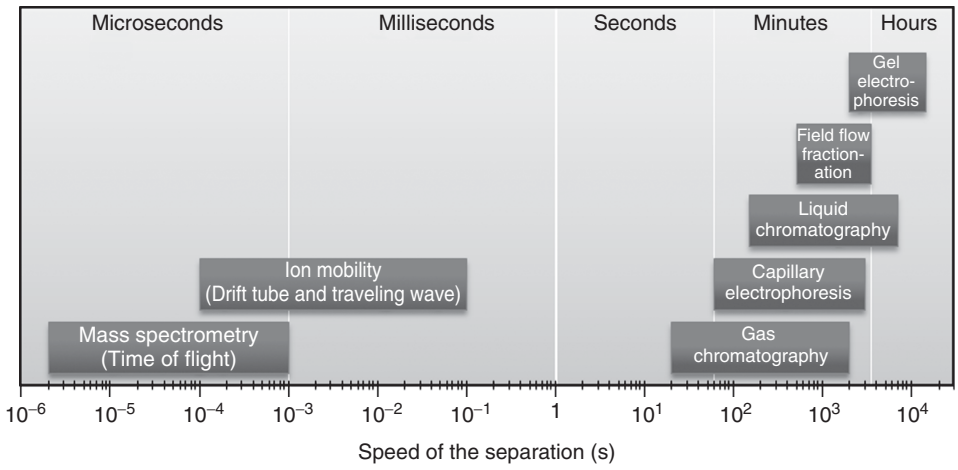

Figure 9.5 Speeds for separation techniques commonly applied to metabolite analysis. Note the logarithmic scaling on the abscissa.

TABLE 9.2 Several Single-Dimensional Separation Methods and the Parameters for Separation, Peak Capacity (ϕ), and Peak Production Rate (ϕ/s)

Separation Method (Single Dimension)	Separation Parameter	Peak Capacity (ϕ)	Signal Peak Production Rate (ϕ/s)	References
Capillary electrophoresis (CE)	Electrophoretic mobility	1000	0.5	62
Gas chromatography (GC)	Vapor pressure	75	6	63
Liquid chromatography (LC)	Hydrophobicity	60	0.06	64,65
Ultrahigh pressure LC		300	0.2	
1D slab gel electrophoresis	Isoelectric point, mass	100	0.03	66
Field-flow fractionation (FFF)	Hydrodynamic radius	10	0.005	66,67
Ion mobility (IM)	Gas-phase cross section	5	500	10

which adds multiple hours in addition to the separation [55,57–61]. While the orthogonality and peak capacity of IM-MS remains comparable to other two-dimensional methods, it is the high speed of IM-MS that affords a great analytical advantage in terms of the peak production rate, as illustrated in Table 9.2 [9].

In addition to the high data production rate of IM-MS, another key advantage of coupling time dispersive IM and MS is the complimentary nature of the timescales for each dimension of analysis. Because time dispersive IM separations (millisecond) occur two to three orders of magnitude slower than time dispersive MS (microsecond), hundreds of mass spectra can be acquired for each IM spectrum, which benefits the sampling resolution across the IM dimension [68]. This temporal sampling benefit is commonly exploited in LC-MS where each LC trace is sampled numerous times by the MS. One criticism of LC-MS has been that the immensely disparate timescales (six to eight orders of magnitude) ineffectively utilize the throughput of the mass spectrometer. IM readily bridges this time disparity between LC and MS, which has been demonstrated for the three-dimensional analysis of complex biological samples [69]. CE-IM-MS has also been demonstrated to improve the sensitivity of MS for low abundance analytes in complex biological samples [70]. Combining front-end separation techniques with IM-MS is still an emerging area that holds great promise for improving the chemical analysis for both contemporary and future applications.

9.3.4 Information Content of Ion Mobility–Mass Spectrometry Analyses

One favorable consequence of the data correlation between IM and mass is the potential for extracting additional information regarding specific analytes within the sample.

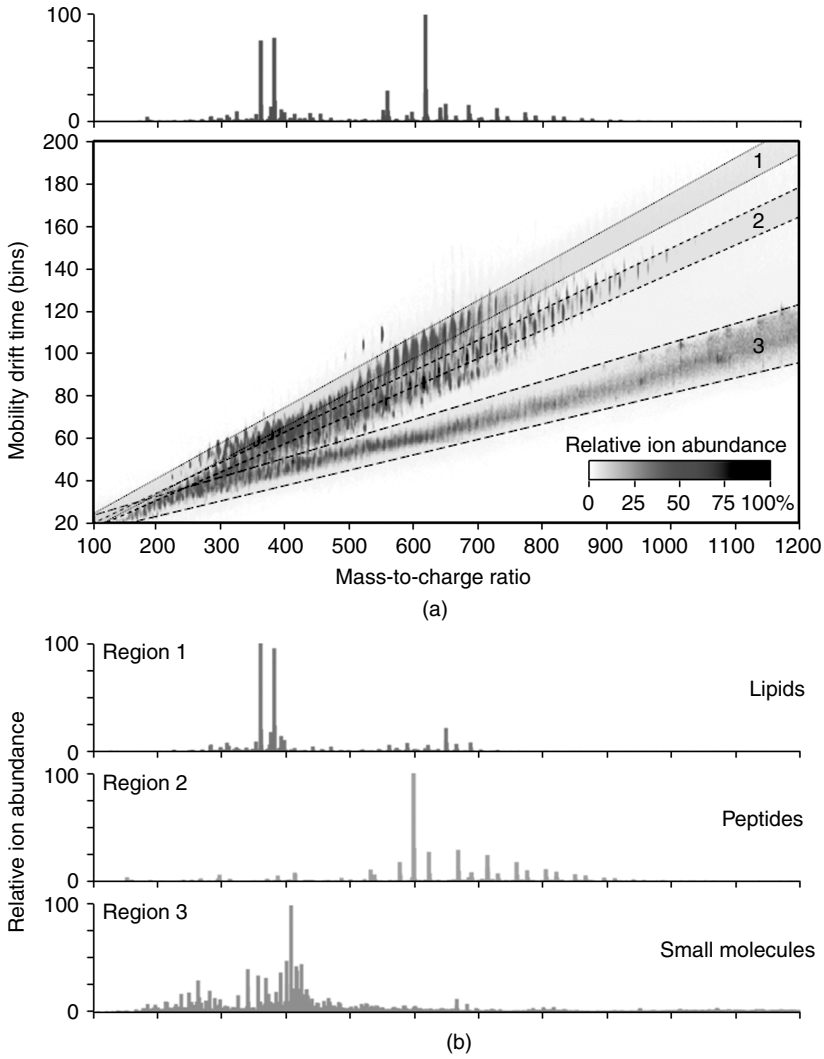

Figure 9.6 (a) Shown is a nanoelectrospray ionization–ion mobility–mass spectrum of a methanol extract of rat blood. Mass-to-charge ratio is depicted on the abscissa, with mobility drift time represented on the ordinate (ms). Peaks are false colored to illustrate relative abundance, with the scale displayed in the inset. Shown above the IM-MS plot is the corresponding mass spectrum for the entire sample. Regions corresponding to various mobility–mass correlation lines are highlighted and labeled, with the extracted mass spectra displayed in (b). Preliminary identifications are assigned based on conformational space occupation. (See color insert.)

This complimentary information can be used in a number of ways for more confident identification and characterization of analyte signals within the sample. Figure 9.6a contains a two-dimensional projection of IM-mass data for a nanoelectrosprayed sample of a methanol extract of rat blood. This data was obtained in <1 min in the authors' laboratory using a traveling wave IM-MS instrument. The spectrum displays m/z (Da) on the abscissa and drift time (ms) on the ordinate. The spectrum is false colored

(heat mapped) to represent relative signal abundances and is composed of almost exclusively singly charged ions due to the nano-ESI conditions utilized. Immediately obvious is the close correlation between the mobility and mass, which manifests as a clustering of data along a mobility–mass correlation line [71]. This results from the intuitive scaling of the analyte size with its weight. Figure 9.6b illustrates the advantage of selective interrogation of data corresponding to the mobility–mass correlation for lipids, peptides, and small molecules. The individual mass spectrum is extracted directly from the combined mobility–mass spectra (Fig. 9.6a). The result is the extraction of relevant analyte signals, based on the distillation of chemical noise, which greatly improves the amount of information that can be gleamed from the data. This strategy has recently been shown to be applicable to metabolite identification for the IM-MS analysis of human blood [72], rat lymph fluid [73], and *Escherichia coli* extracts [74].

One of the challenges of metabolite studies is the diverse representation of chemical species classified as metabolites. These include both large (e.g., peptides, lipids, and glycosides) and small (e.g., alkaloids, terpenes, and phenols) biomolecules, but traditionally focused on the latter because of the available technologies for small-molecule analysis (e.g., GC-MS). As described previously, the IM-MS analysis provides an advantageous dimension of information in the form of molecular class-specific mobility–mass correlation lines, which can be used for identification purposes and also for isolation and extraction of certain chemical signals. From a global analysis perspective, this has the advantage of interrogating a large amount of chemical data simultaneously, which is particularly advantageous for whole systems approaches [75]. As an example of the utility of IM-MS for whole systems studies, Fig. 9.7a depicts a mapping of ion collision cross section (IM) versus ion mass for a large sample

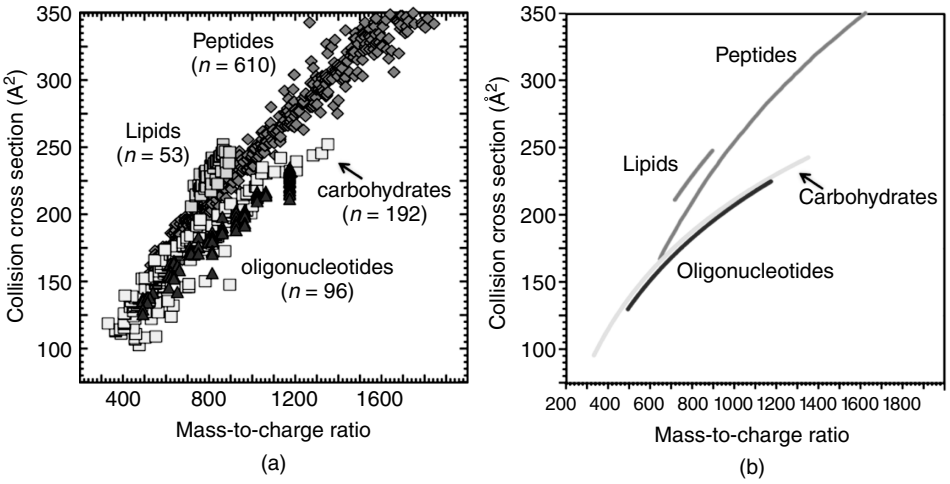

Figure 9.7 (a) The mass-to-charge values and calculated collision cross sections (in Å^2) obtained using a drift tube ion mobility–mass spectrometer for 53 lipids, 610 peptides, 192 carbohydrates, and 96 oligonucleotides, illustrating the separation of biomolecular classes in two-dimensional space using IM-MS. (b) Logarithmic regression fits applied to the biomolecular class distributions in (a). *Source*: Adapted from Fenn LS, Kliman M, Mahsutt A, Zhao SR, McLean JA. Anal Bioanal Chem 2009;394:235. Fig. 9.1(a,b), with kind permission from Springer Science+Business Media. (See color insert.)

number ($n = 951$) representing four biomolecular chemical classes: lipids, carbohydrates, peptides, and nucleotides. Figure 9.7b contains logarithmic regression fits of the data sets in Fig. 9.7a and illustrates the average mobility–mass correlation for each biomolecular class. Note that the IM data normally projected as drift time has been converted to collision cross section (in $Å^2$). What is immediately obvious is that the average mobility–mass correlation is different for each class, with notable disparity between peptides and carbohydrates. The data projections in Fig. 9.6 represent an initial attempt at mapping mobility–mass data space for a large data set representative of several molecular classes. The ultimate goal of such a project is to generate a reliable "atlas" of conformational space of biologically relevant molecules and apply this *a priori* knowledge toward an integrated "omics" approach [76].

9.4 TANDEM METHODS FOR ION MOBILITY–MASS SPECTROMETRY/MASS SPECTROMETRY

IM has so far been discussed with regard to the analytical merit of performing fast separation of mixtures and using the resulting separation to locate relevant chemical signals that may otherwise be disregarded in a single dimension of mass analysis, such as the case of structural isomers. While the identification of significant analyte signals (markers) in the spectrometric analysis is certainly important, it is more challenging to then assign these signals a chemical identity [77]. Tandem MS experiments (MS/MS) are a critical aspect of MS approaches to metabolite identification. MS/MS involves two mass separation steps with ion fragmentation occurring in between. The first MS is used to isolate one mass to charge, which is then subjected to a fragmentation step (most commonly through energetic gas collisions in a pressurized chamber), causing decomposition of the selected precursor ion into fragments. The fragment ions are then analyzed in a second MS stage to generate a fragment ion mass spectrum. Fragmentation data is used to determine the precursor ion's identity based on predicted fragmentation pathways [78]. Additionally, there are several useful "scans" that can be utilized with MS/MS in order to monitor, for example, specific product ions that are known metabolic markers for a particular drug. These tandem strategies are briefly outlined later in this chapter. Tandem MS experiments are performed using either serial stages of MS instrumentation (tandem in space) or an ion trap instrument (tandem in time). When we discuss tandem experiments in IM-MS, we have dealt only with tandem in space arrangements (primarily as a result of the lack of development of ion-trapping-based IM technologies, although it is noted that such a device has been suggested theoretically [79]).

9.4.1 Scanning Modes for Tandem Mass Spectrometry

There are several "scan modes" that are useful in multistage tandem MS for metabolite screening applications. These modes differ only in whether each MS stage is operated in narrow (selected MS) or broadband (full MS) mode. With two MS stages (including a fragmentation stage in between), there are four possible combinations of scan modes that can be utilized. Figure 9.8 illustrates these four scan modes, namely, (a) precursor ion scan (full–selected), (b) neutral loss scan (full–full), (c) product ion scan (selected–full), and (d) selected reaction monitoring (selected–selected). Traditionally, these modes were applied to triple quadrupole instruments, but the general methodology

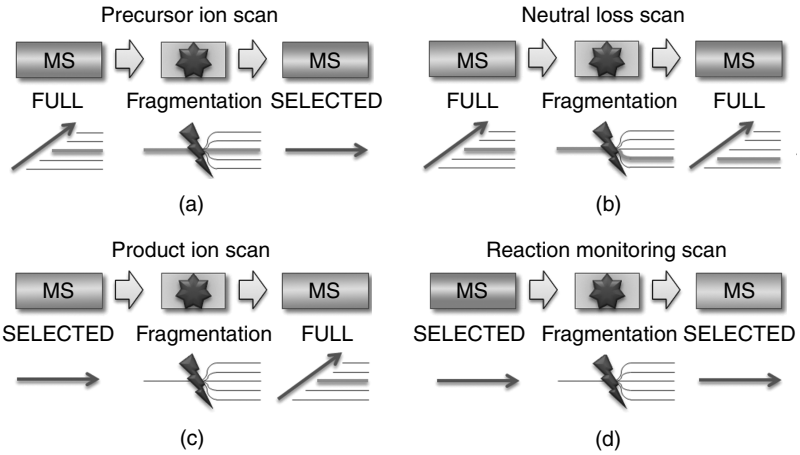

Figure 9.8 Illustrations of the various MS/MS scanning methods for two tandem MS stages. Fragmentation occurs between the two mass analyzers, as depicted. (a) A precursor ion scan, where the first mass analyzer performs a full scan, while the second mass analyzer is fixed to a given m/z that corresponds to an expected product ion. (b) A neutral loss scan, where both mass analyzers perform a full scan, with the second mass analyzer set to a fixed mass-to-charge difference from the first. Commonly, the analyzer offset corresponds to a known biotransformation. (c) A product ion scan in which the first mass analyzer isolates a particular mass-to-charge value and the second mass analyzer performs a full scan to obtain all resulting fragments. (d) A reaction monitoring scan in which the first mass analyzer selects a specific mass-to-charge value and the second mass analyzer is set to selectively monitor for the appearance of a particular product ion.

has since carried over to other MS/MS instrumentation, such as Q-TOF (quadrupole time of flight), TOF–TOF, and, to some extent, various ion trap platforms. The underlying difference in applying these scan modes to different instrument platforms is in the resulting performance: TOF instruments generally sacrifice sensitivity and broad-range linear response (e.g., quantitative ability) for high speed and high resolution/mass accuracy. Ion traps trade some degree of performance (speed and mass resolution) for experimental versatility; most notably, the capability to do multiple stages of tandem experiments (MS^n, where $n > 2$). Because TOF instruments obtain a broadband mass spectrum, the precursor and selective reaction monitoring experiments are performed post data acquisition. The following is a brief description of each scan mode as the modes are applied in tandem MS/MS experimentation.

The precursor ion scan (Fig. 9.8a) and neutral loss scan (Fig. 9.8b) modes are the first tandem MS experiments that are utilized in a metabolite study. These MS/MS experiments are considered as reasonable preliminary methods since the information gained is broadly encompassing and requires very little *a priori* knowledge regarding the system under investigation. In a precursor ion scan, an expected product ion is selected with the second MS stage, and the first MS stage is scanned through a range of m/z values until the expected product ion signal appears. The product ions of interest represent common metabolic alterations, such as glucuronidation ($M + 176$). Additionally, the second MS stage can be set to monitor the m/z of the parent compound itself in order to discover metabolites that consist an unaltered portion of the

parent compound, such as oxidation (M + 16). Because the fragmentation step in an MS/MS experiment results in both fragment ions and fragment neutrals, the mass spectrometer is blind to the latter. To overcome this fundamental limitation, the two MS stages can be set to transmit a fixed m/z difference from one another, representing a characteristic neutral loss. For example, to locate an oxidation product metabolite, the first MS stage is scanned through a range of m/z values and the fragment ion, M − 16, is monitored by the second MS stage. This will find all precursor ions that have been oxidized.

The product ion scan (Fig. 9.8c) is the conventional mode for MS/MS experiments. In a product ion scan, a precursor ion m/z is isolated and transmitted by the first MS stage, fragmented, and the entire fragment ion spectrum is obtained in the second MS stage. The product ion scan is useful for characterizing a precursor ion once it has been identified as being of interest. Finally, the reaction monitoring experiment (Fig. 9.8d) is a powerful and specific mode of MS/MS operation used primarily when quantitative information is sought. In this mode, an m/z of interest is selected by the first MS, fragmented, and a specific m/z is monitored by the second MS. Prior knowledge regarding the expected product ions is necessary. A single or multiple set of fragment ion m/z values can be monitored for each precursor ion transmitted by the first MS.

As mentioned previously, these scanning modes were originally developed for triple quadrupole instrumentation, but recent advances in hybrid MS instrumentation has expanded their usage to other platforms. The Q-TOF configuration is a particularly important instrument platform which replaces the final MS stage of a triple quadrupole with a broadband TOF MS. This has the significant advantage that TOF MS performs with greater analyte sensitivity in its conventional broadband MS mode than a quadrupole operating in a full-scan MS mode. The other important analytical advantage that Q-TOF instruments hold over a triple quadrupole platform is higher speed and, consequently, higher sample throughput. Finally, the high mass resolving power capabilities of TOF greatly improve the mass measurement accuracy. Contemporary Q-TOF instruments are capable of resolving powers in excess of 40,000, with a theoretically unlimited mass range. Macromolecules in the MDa (10^6 mass units) range have recently been studied using Q-TOF instruments [80].

9.4.2 High Performance Tandem Ion Mobility–Mass Spectrometry Modes

The addition of IM to multistage MS instruments offers greater experimental flexibility with regard to tandem experiments. For example, the use of an FAIMS device at the front end of an MS instrument introduces an added dimension of chemical noise discrimination that serves to enhance important ion signals appearing at low abundance [81]. A more versatile example is the addition of an IM dispersive stage into a hybrid Q-TOF instrument, which creates the useful MS-IM-MS configuration (specifically, a Q-IM-TOF). Because of the necessary transition between the two vacuum MS stages into the pressurized IM, elevated pressure ion guides are utilized between each stage to efficiently transfer ions into and out of the IM device, which creates an advantageous addition of two collision cells in the MS-IM-MS instrument configuration. Thus, collisional activation of ions can be accomplished before or after the IM separation, which creates a number of useful tandem arrangements. The next section deals with the various tandem configurations afforded by the MS-IM-MS configuration,

and we consider some of the added information that can be gleamed from each stage of tandem analysis. Regarding nomenclature, the hyphen designates two coupled analyzers (e.g., IM-MS) while the forward slash designation (e.g., MS/MS) denotes tandem capabilities (i.e., a collision cell between the two components).

When considering an analytical configuration that brackets a mobility separation between two mass analyzers (MS-IM-MS), the first possibility is to select an m/z window with the first MS stage and immediately induce fragmentation before the IM-MS analysis (i.e., MS/IM-MS). This will generate a comprehensive 2D IM-MS spectrum of resulting fragment ions (Fig. 9.9a). This strategy is identical to performing a product ion scan, but with the added dimensionality of the IM separation, resulting in separation of product peaks based on both mobility and mass-to-charge ratio. The practical benefit is the separation of product ions in chemical space, allowing for the distillation of the fragmentation spectrum. However, isobaric species will not be separated before fragmentation, and the resulting product ion spectrum may reflect multiple isobaric species not easily differentiated.

An alternative mode of operation is to first select an m/z of interest, mobility-separate, and then fragment the ions following the IM region (i.e., MS-IM/MS). As a result, a particular mass of interest is dispersed based on mobility before fragmentation and secondary mass analysis. An example spectrum is contained in Fig. 9.9b. Because ion fragmentation follows the IM separation, the resulting fragment ions will have mobility drift times correlated to their precursor ion signal. Thus, this method allows for the initial separation of isobaric ions based on their different mobilities, while simultaneously correlating the product ions to their precursors. Thus, both chemical noise and isobaric signal overlap are attenuated through this fragmentation method. The MS-IM/MS method can greatly simplify analyte identification based on the removal of contaminating product peaks contributed by isobaric ions.

A third mode of operation that can be utilized in the MS-IM-MS configuration is to isolate an m/z in the first MS stage, induce dissociation, mobility-separate the resulting fragments, and finally, fragment once again before the secondary mass analysis (e.g., MS/IM/MS). This mode of operation (Fig. 9.9c) results in mobility-correlated secondary product ions. Figure 9.10 contains tandem data for the IM-MS analysis of the small carbohydrate, lacto-*N*-fucopentaose 1 and illustrates two modes of tandem IM-MS. In Fig. 9.10a, the ion fragmentation occurs before the IM-MS analysis, which results in a two-dimensional spectrum of fragment ions separated in both mobility and mass space. In Fig. 9.10b, ions are induced to fragment both before and immediately following the IM analysis, resulting in a second stage of fragmentation (a pseudo-MS^3 experiment). In the latter example, the secondary fragments align themselves to the precursor ion signals in which they originated since the IM separation occurs before their formation. The novel aspect of this mode of tandem experimentation (MS/IM/MS) is that simultaneous ion activation can be achieved, generating a complete precursor and fragment ion spectrum during each experimental measurement cycle.

9.4.3 Advantages of High Mass Accuracy and High Resolution in Metabolite Identification

In drug metabolism studies, the molecular formula of a metabolite of interest is a key piece of information. This information is most easily obtained by measuring the exact mass of the ion to high accuracy. High resolution MS can obtain a mass measurement

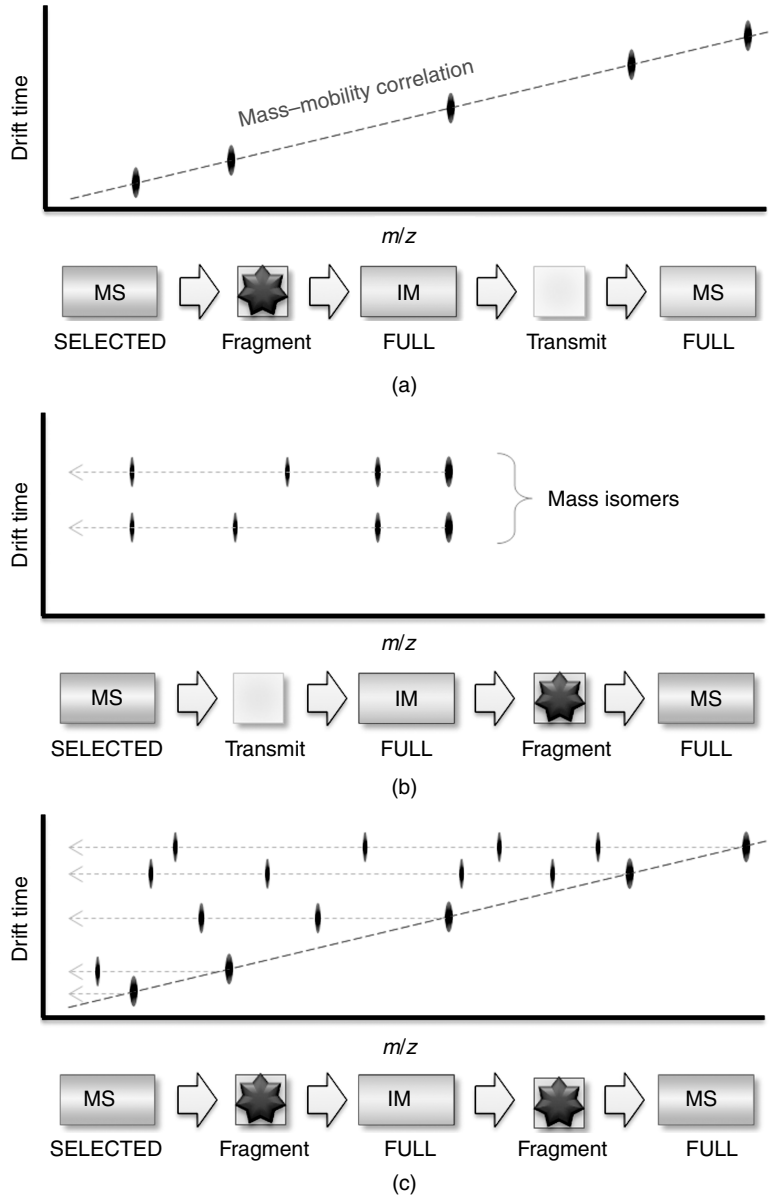

Figure 9.9 Three different tandem modes for MS-IM-MS instrumentation. Each two-dimensional spectrum corresponds to a single m/z value isolated by the first MS stage. (a) MS/IM-MS mode in which fragmentation precedes the IM-MS analysis. (b) MS-IM/MS mode in which the mobility spectrum is generated before ion fragmentation, resulting in alignment of the fragment ions to their precursor ion's measured mobility value. As illustrated, this tandem mode is particularly useful for isobaric ions that cannot be differentiated by MS alone. (c) MS/IM/MS mode in which an isolated m/z value is fragmented both before and after the ion-mobility analysis, resulting in parallel fragment ion alignment, correlating to their respective precursor ion signals. In these examples, only singly charged ions are considered.

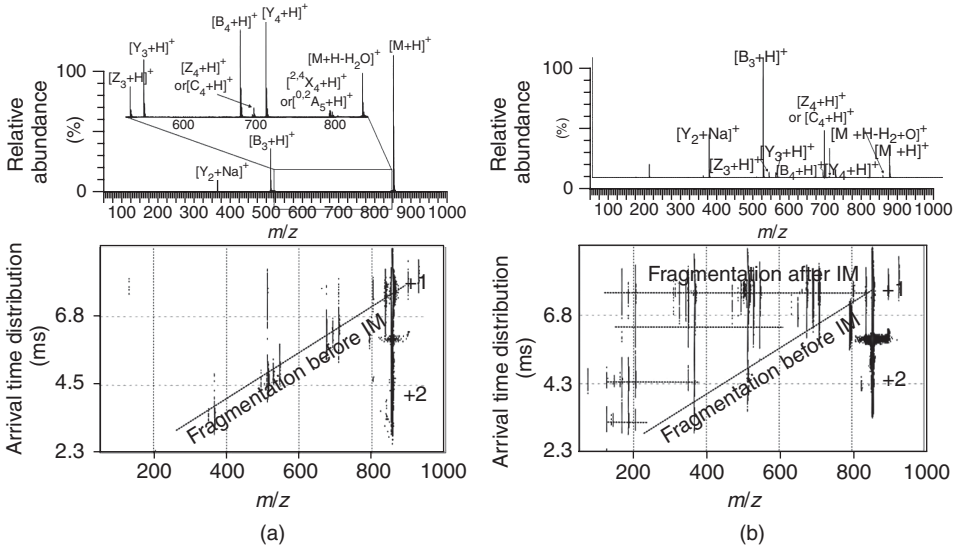

Figure 9.10 Ion mobility–mass spectra of the small carbohydrate, lacto-*N*-fucopentaose 1, illustrating two tandem IM-MS modes. (a) Spectra from fragmentation before IM-MS analysis, which results in dispersion of fragment ion signal across both dimensions of data analysis. (b) Spectra resulting from two stages of fragmentation, one before the IM analysis and one immediately following the IM. In the latter case, fragment ion signal is dispersed across the mobility dimension and secondary fragment ion signals align isobaric in mobility, resulting in simultaneous fragmentation of all ions resulting from the first stage of activation. *Source:* For the mass spectra (in both (a) and (b)), the carbohydrate nomenclature used is from Domon and Costello [82]. Adapted from McLean JA, Fenn LS, Enders JR, Mass Spectrometry Imaging, Methods in Molecular Biology 656, Rubakhin, SS, Sweedler, JV (eds.) 2010. Fig. 21.7, with kind permission from Springer Science+Business Media.

with high precision, which factors directly into the accuracy of the mass measurement. By obtaining an accurate mass measurement with high confidence, one can narrow down the number of possible molecular formulas pertaining to the measured mass.

When utilizing high resolution MS, an approach has been applied to metabolite identification that relies primarily on high mass accuracy to query the entire sample post separation [83]. This method is termed MS^E and functions by initially acquiring data over a large mass range (ca. 100–1000 Da). Subsequently, collision energy is imparted to the same range of ions in a ramped method from low to high energy. This results in data that combines the full scan with corresponding fragments. Using the high mass accuracy and drift time separations of the instrument, product ions can be correlated back to their respective precursor ions based on mass defect and mobility. This resolves the issue of discarding ions on the basis of selective fragmentation, as this method allows for the fragmentation of all ions within a given time range. High mass accuracy is maintained through the use of an internal standard for proper calibration. What results is the ability to perform broadband fragmentation and monitor product ion transitions, as commonly done in scanning experiments, over an entire mass range. A fundamental limitation lies in the fragmentation of isobaric species that are inherently present in complex samples, as product ions for both isobars will be present in the fragmentation spectrum, resulting in convoluted data sets. This is commonly addressed

by performing a preionization separation. Fortunately, the IM separation can distinguish isobars in the mobility dimension. As a result, an additional degree of discrimination is present in compiling a list of product ions for a given precursor.

Tandem MS inherently has limitations. Most notably, this method is insensitive to low intensity precursor ions, as these are often overlooked by automated systems if a particular peak is transient in nature. Selected precursor ions must be well above the limits of detection for a given tandem platform in order to generate meaningful tandem spectra. Isobaric species, which would commonly convolute tandem spectra, may be separated using IM post ionization separations to resolve isobars. While IM-MS enhances tandem experiments, currently, IM separations are insufficient for the routine resolution of structurally similar isobars, for example, compounds that differ by a single chiral center. This is where high resolution structural measurements, such as NMR, are required. However, these methods allow for the relatively rapid interrogation of complex samples, allowing distillation of robust data sets to significant analytes.

We began this chapter by stating the technological challenges of metabolite analysis are substantial. While this is accurate, the number of metabolites expressed by eukaryotes is small in comparison to the number of proteins, which is estimated somewhere at ca. 200,000 for humans. If IM-MS and related technologies are to be commissioned into the global "omics" initiatives, then there is perhaps no greater potential for impact than in metabolite analysis. Future work should seek to implement the real-time acquisition of metabolic information to better represent the dynamic nature of the metabolome. The temporal resolution necessary to understand transient biological responses will likely require the ability to acquire spectra at a rate that exceeds our current analytical capacity using preionization separations. This conundrum lends itself strongly to the case for IM-MS as a means of rapid analysis of drug metabolism. By coupling IM-MS with microfluidics that allow for the trapping and maintenance of cell colonies, a global profile of a microenvironment can be acquired with high temporal resolution. This permits a more complete understanding of the underlying metabolic processes that preclude particular metabolites, while potentially circumventing the dilution issue commonly associated with bulk cultures. Although many technical issues exist with coupling microfluidic environments directly to IM-MSs (e.g., ion suppression by non-informative, high abundance metabolites, and salts), these are surmountable and have been addressed in a modular fashion.

9.5 SUMMARY

IM spectrometry is the separation of gas-phase ions on the basis of differential velocities through an inert buffer gas under the influence of an electric field, this rate being termed *mobility*. Four main IM separation methods exist (drift tube IM spectrometry, traveling wave IM spectrometry, high field asymmetric waveform IM or DMS, and differential mobility analyzer), each utilizing a different approach for separations of gas-phase ions. These methods, although present in various geometries and grounded in disparate physical principles, have the common thread of performing a postionization separation based on mobility. Drift tube and traveling wave IM temporally separate ions along the same space, which has the advantage of generating a broadband spectrum for each measurement cycle, while limited to separating discontinuous

ion pulses. High field asymmetric waveform IM and differential mobility analyzers separate ions along different spatial trajectories, allowing for a continuous beam of ions to be transmitted, which possess a narrow band of mobilities. The latter techniques must be scanned across an instrument parameter (a voltage) to generate a broadband IM spectrum.

Each IM separation method has been integrated with MS for two-dimensional separations based on both mobility and mass-to-charge ratio. These two analytical methods are readily coupled (IM-MS), as both separations occur post ionization, and result in rapid separations occurring on the order of milliseconds and microseconds, respectively. This temporal disparity allows for hundreds of mass spectra to be obtained for each mobility spectrum. For high performance MS applications, broadband dispersion mobility separations are commonly implemented, which allow for global metabolome profiles to be obtained. Because the ionic mobility is a function of gas-phase size and a function of mass, a two-dimensional IM-MS analysis results in a clustering of data along a narrow region of mobility and mass separation space. Thus, the degree of orthogonality between IM and MS is low; however, the rate at which information is generated is very high, with signal peak production rates ca. two orders of magnitude greater than other analytical separations. The correlation between mobility and mass data exhibits a dependence on intramolecular forces, monomer subunit composition, and degree of branching, which allows for the preliminary determination of biomolecular and metabolite class based on mobility. IM-MS allows spectra acquisition temporal resolution on the order of milliseconds, dispersion of chemical noise in two dimensions, separation of isobaric species based on structural differences, and preliminary determination of molecular class based on mobility. IM-MS is also readily coupled to any liquid-phase preionization separation through ESI.

The ability to perform fragmentation between separations in an IM-MS instrument allows for identification studies (tandem experiments) that enhance precursor ion identification confidence. Fragmentation is commonly prefaced with a mass selection step using a scanning mass analyzer (e.g., a quadrupole MS) in order to isolate a single mass-to-charge window. Fragmentation can occur either before (MS/IM-MS) or after (MS-IM/MS) the IM separation, resulting in product ions or isobaric precursor ions separated in mobility space, respectively. Fragmentation before mobility separation results in mobility-resolved product ions, and these product ions can be further fragmented after mobility separation (MS/IM/MS), resulting in secondary product ions that can be correlated to primary product ions on the basis of the congruent mobilities. Broadband fragmentation can also be performed on relatively simple samples using a large mass window (ca. 100–1000 Da), a ramped fragmentation energy profile, and a high performance mass spectrometer. In the latter example, product ions are correlated to precursor ions based on mass defect. Increased confidence is obtained through the use of mobility correlation to this broadband fragmentation method, as isobaric ions will convolute data otherwise.

ACKNOWLEDGMENTS

Financial support for this work was provided by the National Institutes of Health (1 R01GM092218-01 and RC2DA028981), the US Defense Threat Reduction Agency

(HDTRA-09-1-0013), Vanderbilt University College of Arts and Sciences, Vanderbilt Institute of Chemical Biology, and Vanderbilt Institute for Integrative Biosystems Research and Engineering. We also thank Richard M. Caprioli (Vanderbilt University, Department of Biochemistry) and the Vanderbilt University Mass Spectrometry Research Center for use of the Synapt HDMS.

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