

Chapter

Ion Mobility Mass Spectrometry: Instrumentation and Applications

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Abstract

The integration of ion mobility spectrometry with mass spectrometry (as an IM-MS hybrid instrument) provides additional analytical separation and identification capabilities that have greatly advanced various fields, including biomedical, pharmaceutical, and forensic sciences. In this chapter, a comprehensive exploration of various IMS instrumentation platforms is discussed, including Drift tube (DTIMS), Traveling wave (TWIMS), Trapped (TIMS), Field asymmetric waveform (FAIMS), and Differential mobility analyzer (DMA). Their respective advantages and limitations are evaluated in the context of distinct applications, including isomer separation, signal filtering to increase signal-to-noise ratio, and collision cross section (CCS) measurements in targeted and untargeted omics-based workflows. The scanning rate compatibility between various IMS devices and different mass analyzers resulted in various IM-MS hyphenation platforms. Higher sensitivity and selectivity are further achieved with the introduction of tandem IMS such as TIMS-TIMS-MS. IMS separations occur in the millisecond range and can therefore be easily incorporated into the liquid chromatography-mass spectrometry workflows and coupled with ambient ionization MS for metabolomics, lipidomics, proteomics, etc. The emergence of high-resolution IMS instruments such as Cyclic Ion Mobility Spectrometry (cIMS) and Structures for lossless ion manipulations (SLIM) is also discussed for the improvement of separation of isomers and increased predictive accuracy of CCS by machine learning models.

Keywords: ion mobility mass spectrometry, CCS experimental measurements, CCS prediction models, ambient ionization, omics

1. Introduction

Compounds with the same mass-to-charge ratio (m/z) but differ in configurations and conformations exhibit significant variations in potency, pharmacodynamics, and toxicology [1, 2]. In metabolomics, lipidomics, and proteomics studies, the existence of isomers poses significant challenges to effective separation. Analytical methods for isomer separation and characterization include chromatographic separation and differences in tandem mass spectrometry (MS/MS) fragmentation patterns. However, similar retention behavior during chromatography and fragmentation patterns in

mass spectrometry techniques reduce the accuracy of both qualitative and quantitative structural analysis of compounds [3, 4]. Ion mobility spectrometry (IMS) is an effective solution for addressing the intricate structural challenges presented by isomers in omics studies. In addition to isomer separation and signal filtering, IMS facilitates the annotation of features via CCS values thereby enhancing the identification of compounds in non-targeted omics analysis that share common properties (e.g., tandem mass spectrometry (MS/MS), retention time (RT), m/z) [5]. This chapter focuses on the commercially available IMS instrumentation platforms and their integration with other separation methods, sample introduction, and detection techniques for all-omics studies. Furthermore, the applications of computational and machine learning models for the calculation and prediction of CCS are also discussed. Moreover, a detailed examination of novel high-resolution IMS systems, including cyclic IMS (cIMS) and Structures for lossless ion manipulations (SLIM), and their technological advancements is provided.

2. IM-MS: Instrumentation and experimental CCS measurement

IMS separate ions based on their mobility rates (K or K_0 ; K is usually reported as K_0 under standard pressure and temperature) through the combined action of an electric field and neutral buffer gas. The mobility of the ions depends on characteristics of the ions such as mass, size, shape, and charge number, leading to the separation of compounds with the same m/z but different structures (isomers) due to the combined influence of the collisions of the ions with neutral buffer gas and applied electric field [5]. Although IMS can detect the size of an ion, it cannot determine its exact molecular weight. Therefore, IM-MS is more valuable, combining complementary size selection and quality mass separation into a single analysis platform [3]. IMS separations typically occur on a timescale of 10^{-3} – 10^{-2} s, hence, are well-suited for incorporation between LC (10^2 – 10^3 s timescale) and mass spectrometry techniques (10^{-6} – 10^{-4} s timescale), thereby providing three-dimensional orthogonal separation based on polarity, size, and mass of the analytes. This results in a four-dimensional dataset including RT, drift time (DT), m/z , and fragmentation patterns, all captured in a single sampling event [3, 5, 6]. The supplementary advantages of IMS separation when coupled with MS, such as separation of isomers, signal filtering, and CCS fingerprinting, depend on the resolution of the IMS technique and its accuracy of collision cross section (CCS) measurements. These parameters must be considered when selecting IMS instruments. According to separation mechanisms, IMS platforms can be classified into temporal dispersive, spatial dispersive, and confinement and selective release; and based on electric field applications, they can be classified into static and dynamic fields [7, 8]. In temporal dispersive IMS, all ions drift along similar paths colliding with the neutral buffer gas under the influence of electric fields, resulting in different arrival times (mainly DTIMS and TWIMS), while in spatial dispersive IMS, ions are separated along different drift paths (mainly DMS, FAIMS, Aspiration IMS (AIMS), and DMAs). In confinement and selective release IMS (mainly TIMS), ions are trapped within a pressurized region by a precisely adjustable electrodynamic field and are then selectively released based on differences in their mobility [3]. Static field IMS instruments employ linear and constant electric fields (mainly DTIMS and DMA), whereas dynamic field IMS instruments utilize non-uniform electric fields (mainly FAIMS, TWIMS, and TIMS) [8].

2.1 Drift tube ion mobility spectrometry (DTIMS)

A distinctive feature of DTIMS is the uniform application of a weak electric field (10–20 V/cm) that propagates through the drift tube, which is filled with a neutral buffer gas such as nitrogen or helium that has no directional flow, as shown in **Figure 1A**. The two working states of DTIMS are the reduced pressure (~4 Torr e.g., Agilent 6560 IM-QTOF) and atmospheric pressure (760 Torr e.g., TOFWERK-IMS-TOF) [9]. The choice between low-pressure and high-pressure systems involves trade-offs; while low-pressure systems offer higher sensitivity and improved ion focusing due to reduced collisions frequency with the buffer gas, high-pressure IMS systems yield enhanced separation capacity due to more frequent collisions, but often experience significant ion losses [10]. Radiofrequency (RF) confinement in low-pressure DTIMS (3.5 Torr) has been reported for excellent ion transmission resulting in increased sensitivity [11]. The ability to calculate CCS from first principles using the Mason-Schamp equation is perhaps the most significant advantage of DTIMS. The utilization of a low and uniform electric field enables DTIMS to measure K as a primary method, enabling it to calculate the corresponding CCS values. In fact, DTIMS is the only ion mobility paradigm that can provide precise CCS measurement without the need for external calibrants. This is achieved through a method known as step-field, which is the “gold standard” for CCS measurements with a remarkable 0.29% relative standard deviation (RSD) [12]. However, using the step-field method for experimental CCS measurements can be time-consuming and is not practically compatible with the analytical timescale of the chromatographic separation [5]. In contrast, the single-field method (calibrant dependent) is compatible with

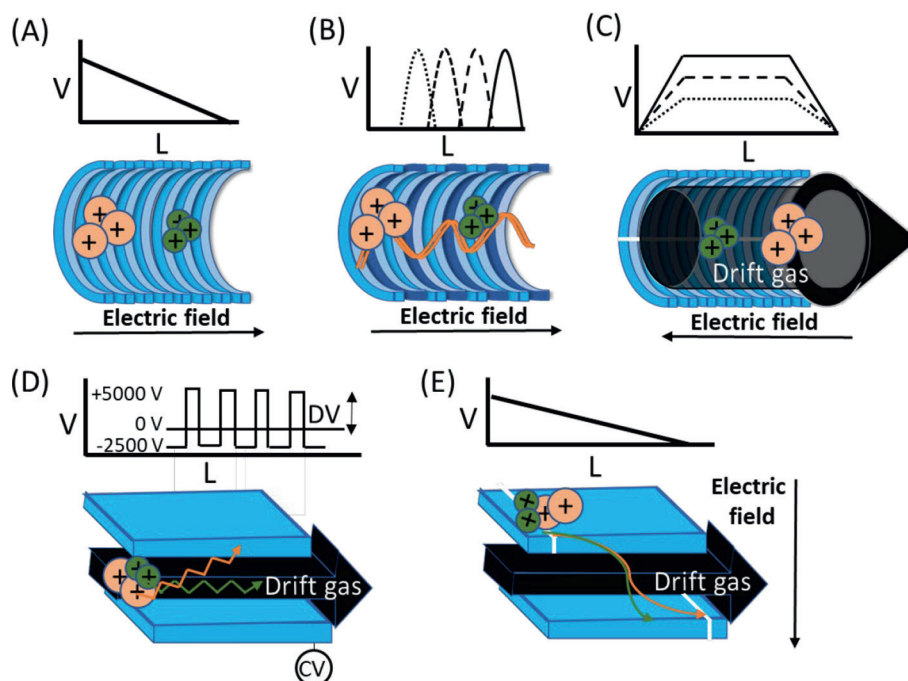


Figure 1. Schematic diagram of various IMS technology and separation mechanisms: (A) DTIMS, (B) TWIMS, (C) TIMS, (D) FAIMS, and (E) DMA.

	DTIMS	TWIMS	TIMS	DMA	FAIMS
Separation mechanism	Temporal	Temporal	Trap & Release	Spatial	Spatial
Gas flow to ion motion	No net flow	No net flow	Parallel	Perpendicular	Parallel
Electric field	Static	Oscillating	Static	Static	Oscillating
Ion injection	Pulsed ion packet	Pulsed ion packet	Variable	Continuous filter	Continuous filter
Ion analysis	All ion analysis	All ion analysis	All ion & scannable	Scannable	Scannable
CCS	Direct	Calibrated	Calibrated	Direct	No CCS
Common calibrants	Agilent tune mix	Polyalanine, Waters CCS Major Mix	Agilent tune mix		
Resolving power	60–80 ^a	~40 ^b	~200	<100	~40 ^c
Vendors	Agilent, ToFwerk, Excellims	Waters	Bruker	SEADM, TSI	Owlstone, Thermo, Heartland, Sciex
Mass analyzers	TOF, Quadrupole	TOF	TOF, FTICRs, Orbitraps	TOF, FTICRs, Orbitraps	TOF, FTICRs, Orbitraps

^a180–250 with HRdm.^bcIMS and SLIM: >400.^cUp to 500 with the doping of the N₂ carrier gas with light gases such as He or H₂.

Table 1.
General information on each IMS technology.

chromatographic separation. It utilizes calibrant ions to produce highly reproducible CCS values with 0.54% RSD compared to the step-field method [12]. Other IMS platforms (e.g., TWIMS) use this single-field method using calibrants that have well-characterized CCS values previously determined with DTIMS instruments to create a calibration curve for CCS measurements of unknown analytes [5, 13, 14]. Calibrant compounds used in DTIMS include the Agilent Tune mix (**Table 1**).

While DTIMS systems enable comprehensive ion collection, i.e., the arrival time of all ions is recorded during each measurement cycle, particularly valuable for the analysis of complex biological samples, ions are not continuously injected. Instead, ion packets are introduced as ion pulses into the drift tube using an ion gate [15] or an ion funnel [16]. The time scale of the ion release time and IMS separation time is 25–400 μ s and 25–100 ms respectively, resulting in an experimental duty cycle of ~0.04–1%. Consequently, only a fraction (0.1–1%) of the generated ions are transmitted to the drift tube for separation, resulting in a low sensitivity [17]. To improve the DTIMS sensitivity, an ion multiplexing approach is utilized, involving the pulsing of multiple packets of ions into the drift region at defined times. The multiplexing strategy results in an overlapping ion mobility spectrum, which is subsequently deconvoluted to the correct arrival times using schemes such as the Hadamard Transformation algorithm, achieving improvements as high as 50% duty cycle [5, 18]. An additional challenge of DTIMS systems is the low resolving power which can be improved using

a post-acquisition data reconstruction technique or modifying the DTIMS instrumentation [5, 19]. An example of a reconstruction technique is the High-resolution demultiplexing tool (HRdm, Agilent Technologies), which effectively increases the resolving power from ~60 to a range between 180 and 250 [20]. This involves passing multiple ion packets through the drift tube, giving each ion packet a shorter accumulation time to improve the duty cycle while reducing the negative effects of space charge. The spectrum obtained is then deconvoluted and demultiplexed using the Hadamard transform algorithm, resulting in an improved signal-to-noise ratio and lower detection limits. The increase in the resolving power through instrumentation is accomplished by increasing the voltage drop across the drift cell and decreasing temperature [5]. For precise DTIMS measurements, it is essential to keep the ions in the low field limit. Therefore, to increase the voltage drop either the length of the drift cell or pressure needs to be increased [5, 21]. Examples include DTIMS systems of 2 m by Bowers et al. [22] and ~2.9 m by Clemmer et al. [23], as well as atmospheric pressure DTIMS systems yielding resolving power between 100 and 250 [9].

2.2 Traveling wave ion mobility spectrometry (TWIMS)

The drift region of TWIMS is very similar schematically to the DTIMS platform; it contains a stacked set of ring electrodes. However, in TWIMS, an RF voltage is applied across consecutive electrodes to confine the ions radially, and pulse differential current (DC) voltage is applied to push ions axially as shown in **Figure 1B**. Similarly to the RF-confining drift cell, the RF confinement in TWIMS focuses the ions, leading to increased analyte signals attributed to reduced ion diffusion. Under the combined actions of the applied voltages, traveling electric field waves are formed to propel the ions through the IMS tube under reduced pressure of ~2–4 Torr. The amplitude and speed of the electric waves determine the ion separation in TWIMS [14]. Due to the existence of RF voltage, ions are not in a constant field in TWIMS mode, requiring prior calibration of TWIMS instruments with ions of known mobility before determining CCS values of unknowns (analogous to the single-field CCS measurements in DTIMS) [5, 14]. Various compounds are used as calibrants in TWIMS, including polyalanine and Waters CCS Major Mix (**Table 1**). Since the accuracy of CCS measurement in TWIMS depends on the type of calibrants being used, class-specific calibrants are needed for more accurate CCS measurements. For example, calibrating instruments to obtain CCS values using peptides in lipid analyses has demonstrated a significant error [5, 24]. TWIMS shares both advantages and disadvantages with DTIMS, such as the full ion mobility spectrum acquisition and pulsed ion packet delivery by ion gating. Two primary advantages distinguish TWIMS: the low voltage requirements due to constant wave heights, and the ability to manipulate ion motion to long path lengths without significant ion losses. These attributes were the key to the development of two platforms with extremely long path lengths leading to very high resolving power: cIMS from Waters Corporation with a resolving power of ~750 for 100 passes [25] and SLIM, developed at PNNL and commercialized by MOBILion Systems (Chadds Ford, PA) with a path length of 13 m in a device measuring 45.9 cm × 32.5 cm, with a separating power of ~1860 [26].

Commercial TWIMS instruments are produced by Waters Corporation, which includes the Synapt G2 and Vion series (**Table 1**). The Synapt G2 model is designed with an IM tube positioned behind the quadrupole. The collision cell is situated both before and after the drift tube (in DTIMS the collision cell can be connected only after the IMS), enabling TWIMS-MS to perform time-aligned parallel fragmentation [3].

The Vion series moved the IM tube and its frontally connected collision cell to the front of the quadrupole. For comprehensive compound structure elucidation, in addition to the collision-induced dissociation (CID), TWIMS can combine surface-induced dissociation (SID) or electron capture dissociation (ECD), essential tools for structural details of both ligand-bound and unbound [3]. Compared to DTIMS, the resolving power of TWIMS is not significantly improved as it is less than 100.

2.3 Trapped ion mobility spectrometry (TIMS)

The separation principle of TIMS is effectively inverted from traditional ion mobility modalities. While DTIMS and TWIMS methods maintain, the essentially stationary gas flow, with an electric field parallel to the ion movement, TIMS utilizes a unidirectional buffer gas parallel to the ion motion, accompanied by an opposing electric field (ca. 70 V/cm) [3]. The TIMS analyzer is comprised of a set of electrodes that form three regions: the entrance funnel, the TIMS mobility region (ion mobility analyzer), and the exit funnel. The entrance and exit regions serve to control ion deflection and focusing, while the TIMS mobility region is utilized to accumulate, trap, and elute ions of interest which effectively increases the measurements' duty cycles. In the TIMS mobility region, different plates along its length modulate the potential of ions as a function of distance. Consequently, ions with larger CCS values will be pushed further along the TIMS analyzer due to the energy they receive from the carrier gas as shown in **Figure 1C**. The accumulated ions can then be eluted from the TIMS analyzer by sequentially lowering the position-dependent plate potential as a function of time. This sequence of accumulation and elution processes can be adjusted to optimize for fast scans (tens of ms) or high-resolution separations (hundreds of ms) [5]. Optimized stepping scan functions can provide IMS resolving power of >300 while reducing overall experiment time and increasing the duty cycle [10]. An additional main difference between TIMS and the previously described DTIMS and TWIMS techniques lies in its operational scanning mode. In DTIMS and TWIMS, all ions are observed under the same experimental conditions, while TIMS requires changes to experimental parameters to elute all ions. Though TIMS instruments can indeed measure K as a primary method (thus obtaining CCS values), calibrating TIMS with analytes of known mobility prior to analysis (similar to TWIMS) is needed [5]. An example of calibrant ions used in TIMS CCS measurement is the Agilent tune mix (**Table 1**).

2.4 Field asymmetric waveform ion mobility spectrometry (FAIMS)

Both DTIMS and TWIMS separate ions by the gas-phase mobility inherent in an ion for a given gas under low electric field conditions. At high electric fields (i.e., > 10^4 V/cm), ion mobility begins to change due to non-elastic ion-gas collisions (e.g., reactive, or interactive ion-gas collisions). An IM technique that exploits this differential mobility behavior at high fields is FAIMS operating at atmospheric pressure and near-ambient conditions [14]. Two ions with different chemical compositions can have the same ion mobility at a low electric field, but completely different mobilities at a high field, allowing them to be differentiated in the FAIMS measurement. FAIMS's current commercial instruments include Thermo Fisher Scientific, Owlstone Medical, Heartland MS, and Sciex (**Table 1**). FAIMS utilizes an asymmetric waveform voltage known as Dispersion Voltage (DV) which comprises high-field components of short duration and low-field components of a longer time. As a result, ions oscillate between two electrodes in an up-and-down motion, as illustrated in **Figure 1D**.

The dispersion voltage alone would ultimately cause all ions to contact the electrodes and be neutralized. To account for this, a second voltage – a variable compensation voltage (CV) is applied during the experiment. For ions at a specific K value, a given CV value enables ions, under the influence of parallel gas flow, to keep a straight-lined trajectory and exit the chamber to the mass analyzer [3]. In fact, FAIMS acts like a quadrupole mass analyzer and in this way contrasts temporal dispersive IMS instruments in which all ions are transmitted simultaneously. FAIMS can be used in two ways: [1] by scanning a range of CVs to create a fingerprinting in global analyses, or (ii) by selecting CVs values related to the analytes of interest for targeted analysis [14]. Due to the challenging nature of characterizing ion mobility behavior under high fields, FAIMS instruments are unable to provide CCS values. Moreover, the ion structure itself can change from low to high field strengths during oscillation. The different mobility behavior in FAIMS may result from the dipole orientation and the clustering and declustering of the ions. FAIMS devices can be fabricated in different geometries, such as cylindrical, planar, and chips [5]. Differential mobility spectrometry (DMS), differential ion mobility spectrometry (DIMS), along with FAIMS operate under the same electronic mechanism, differing primarily in the geometry of their respective electrodes. Furthermore, these devices do not pulse ions into the mobility region as seen in DTIMS, TWIMS, and TIMS [3]. The continuous ion injection enables FAIMS/DMS/DIMS devices to increase the signal-to-noise ratio for the ions of interest by greatly removing unwanted chemical noise in MS spectra.

2.5 Differential mobility analyzer (DMA)

DMA operates similarly to DTIMS; both systems use a constant electric field and can measure CCS directly. However, DMA instruments introduce distinct features, including unidirectional gas flow (as opposed to the no-directional gas flow in DTIMS), operation at ambient pressure, and scanning for target analytes [10]. This scanning capability makes DMA a narrow bandpass IM technique, analogous in concept to FAIMS, where ions travel between two parallel electrodes in the presence of a gas flow. In DMA a constant electric field is applied between two cylindrical and concentric metal electrodes and ions are introduced at the entrance electrode where they are pushed towards the exit electrode through orthogonal sheath gas flow, as shown in **Figure 1E**. Only ions with the appropriate mobility will reach the cell exit, while others collide with the electrode, thus preventing their detection [8]. By scanning the electric field, an ion spectrum based on the different ion mobilities can be recorded. The well-defined nature of the electric allows for high-precision measurements of ion CCS using DMA. Notably, DMA can be utilized for analyses that are not possible with DTIMS; DMA detects very large analytes, such as antibodies, aerosol particles, viruses, and other macromolecules (~ 10 's to 100 's of nm^2); and is not commonly used in screening applications for small molecules (e.g., lipids and metabolites) [5]. DMA instruments are currently marketed by SEADM and TSI (**Table 1**).

3. Ion mobility mass spectrometry hyphenation

3.1 Mass analyzers interfaced with IM

The difficulty of coupling an IM device to a mass analyzer is caused by the need for scanning rate compatibility of both instruments. Time of flight (TOF) mass

analyzers due to their microsecond acquisition timescale are readily hyphenated with all types of IM devices, facilitating the recording of several mass spectra per mobility scan [27]. For higher MS performances in terms of dynamic range, mass resolving power, and mass accuracy, Fourier transform mass spectrometry (FTMS) analyzers (e.g., Orbitrap-based or FTICR instruments) are required. FTMS analyzers can be readily coupled with spatial dispersive IM instruments, for instance, the coupling of FAIMS with LTQ-orbitrap and FTICR instruments is routinely used [14]. Hyphenation of FTICR or orbitrap-based instruments with temporal dispersive IM systems is less straightforward due to the time scale difference between the rapid IM separation and the slow FTMS acquisition rate (up to 1 s for FTICR). To circumvent this limitation, the IM separation is “slowed down” to make it suitable for the FTMS scan rate [14]. Examples include the coupling of DTIMS with an Orbitrap-based instrument using a dual gate approach [28]. The scan rate in TIMS in theory can be conducted at any speed and synchronized with any analyzer. For instance, TIMS coupling with TOF analyzer, FTICR coupling with gated TIMS, selective accumulation-TIMS (SA-TIMS), and oversampling selective accumulation TIMS (OSA-TIMS) [14, 27]. However, TOF mass analyzers are used in all the commercial IM-MS platforms (e.g., DTIMS, TWIMS, and TIMS) due to the complexity nature of coupling with FTMS analyzers.

3.2 Front-end separations coupling with IM-MS

The incorporation of front-end separation techniques such as LC with IM-MS results in an enhanced peak capacity, detection of more features, separation of co-eluting compounds and isobaric components, and increasing S/N ratios due to the orthogonality of the separation techniques [5]. Notably, the addition of IM to LC-MS workflow has been shown to yield a 15% increase in peak capacity, as reported by Pacini et al. [29]. In the context of lipid mapping of human plasma, the implementation of 2D-LC coupled with MS (LC $\times$ LC-MS) enabled the detection of 1100 features, with 100 lipids identified. Comparatively, the LC-IM-MS approach provided 800 features, identifying 55 as lipids. Despite the fewer features detected by LC-IM-MS, it offers better structural identification and higher throughput (190- and 20-min analysis time for LC $\times$ LC-MS and LC-IM-MS, respectively) [30]. Olajide et al. utilized a multidimensional LC-IM-MS/MS method to detect features that served as strain-indicating biomarkers for efficiently discriminating *E. coli* strains [4]. Paglia et al. used LC-IM-MS^E for metabolomic and lipidomic analyses of frontal cortex samples from Alzheimer’s Disease (AD) patients [31]. Other front-end separation techniques coupled with IMS include gas chromatography (GC) as GC-IMS for fingerprinting volatile organic compounds (VOCs) from the feces and urine of AD-model mice [32]. Furthermore, 2D GC (GC $\times$ GC) coupled to IM-MS was used to analyze *Calendula officinalis* plant extracts, with the IM dimension effectively separating isobaric compounds not resolved by the 2D-GC [33]. Capillary zone electrophoresis (CZE) was coupled with TWIMS-MS to resolve glycan isomers [34]. Supercritical fluid chromatography (SFC) and TWIMS devices were coupled to detect and quantify four nonsteroidal androgen receptor modulators from bovine urine samples [14]. The absence of front-end separations leads to severe matrix interferences and ionization suppression which is a challenge to conventional IM-MS [4]. Solid-phase extraction (SPE) coupled with IM-MS is a promising alternative, such as the SPE-IM-MS method proposed by Zhang et al. for

the simultaneous targeted and untargeted measurements of metabolites in complex human fluids [35].

3.3 Ambient Ionization Coupling with IM-MS

Ambient ionization MS is a technique for direct analysis by ionizing analytes at ambient pressure and temperature conditions without sample preparation. The advantages of ambient ionization techniques include minimal sample preparation, low sample and solvent volume utilization, and direct rapid analysis [36]. Ambient ionization can be largely categorized into three main classes primarily based on their desorption methods: liquid extraction, plasma desorption, and laser ablation [37]. An extensive description of each ambient ionization technique under each classification and their applications in forensics, biomedical, environmental, bioanalysis, in vivo analysis, food and agriculture, and reaction monitoring and catalysis have been detailed in previous reviews [37, 38]. The direct sample analysis using ambient ionization MS suffers severe matrix interference and chemical noise due to no chromatographic separation and reduced selectivity as isomers cannot be separated [4].

The coupling of IM-MS to ambient ionization has proven to increase the technique selectivity due to isomer separation [39]. In addition, ambient ionization IM – MS is very rapid compared to classic LC – IM – MS workflows. Desorption electrospray ionization (DESI) was coupled to DTIMS to investigate charge state distributions and cross sections for protein ions in a single experiment [40]. DESI was also coupled with FAIMS to effectively increase the image quality of targeted compounds in sea algae and mouse brain tissue sections [41]. The capability of DESI to produce ions directly from tablets and creams was explored in combination with hyphenated IMS for the analysis of pharmaceutical drugs [42]. The signal filtration capability of TWIMS was demonstrated in a selective surface analysis when coupled with Desorption atmospheric pressure photoionization (DAPPI) and Direct analysis in real-time (DART) resulting in a low limit of detection (LOD) [43]. Liquid extraction surface analysis (LESA) and FAIMS were integrated to image proteins in mouse brain and liver tissue samples [44]. FAIMS has also been coupled with paper spray (PS) to obtain metabolomic and liposome characteristics in breast tissues [45]. Olajide et al. coupled PS and leaf spray (LS) to DTIMS for the separation of geometric and constitutional isomers [39]. Furthermore, the PS-IM-MS/MS developed by Olajide et al. was utilized to distinguish five *Bacillus* species and seven *E. coli* strains [4, 20].

3.4 Tandem ion mobility (IMS/IMS)

The coupling of multiple ion mobility cells allows low-intensity features to be detected. Tandem ion mobility separations have been achieved either by hyphenation of the FAIMS device with conventional ion mobility geometry or by combining ion mobility cells of the same geometry [14]. Examples include FAIMS-DTIMS-MS with the FAIMS increasing the S/N ratio and the DTIMS providing accurate CCS measurement [46], DTIMS-DTIMS-MS [47], and TIMS-TIMS-MS [48]. In a study on the tryptic peptide, IMS provided a peak capacity of 60–80 while tandem IMS provided a peak capacity of 480–1360 indicating the advantage of using tandem IMS in the analysis of complex mixtures, especially in metabolomics, lipidomics, and proteomics [49]. The history of tandem IM instruments, recent developments, and detailed applications to biological systems can be found in a recent review [50].

4. High-resolution ion mobility and new technologies

The development of high-resolution IMS (HRIMS) has emerged as a solution to the low resolving power of conventional IM techniques (~ 60), which limits the separation of difficult isomers such as cis/trans. Achieving a resolving power of at least 300 is considered essential for effectively resolving such challenging isomers [51]. HRIMS involves advancements in technology and techniques to improve the separation and characterization of ions in complex samples. This is particularly important as samples become more complex and require more precise separation methods [52]. IMS platforms that offer high resolving power include Waters' cyclic ion mobility spectrometry (cIMS), MOBILion Systems' SLIM device, and the previously-described Bruker's TIMS [10].

4.1 Cyclic ion mobility spectrometry (cIMS)

cIMS is an innovative platform that uses the TWIMS concept but has a closed-loop design with a 98 cm path length. It is comprised of an IMS separator, ion entry, and ion exit regions. This model utilizes opposite phases of RF voltage to adjacent plates in the y-axis for a pseudopotential barrier and ion confinement in the z-direction. The applied DC voltage on the "repeller" band electrodes gives ion confinement in the x-axis and TWs in a repeated pattern are supplied to the RF electrodes to propel ions forward which gets them separated as a function of their respective mobilities (**Figure 2**). During the injection of ions, the DC bias of the array electrodes is lower than that of the cIM electrodes, the prearray store (prior to the entrance), and the exit array regions. During IM separation, the DC offset applied to the array electrodes is increased to be similar to the cIM electrodes while being lower than that of the entrance and exit regions. Then the ions are guided through the circular path using the TW voltages applied to the cIM and array electrodes and are subsequently separated based on their ion mobility. After the separation period, the mobility-separated ions are ejected (towards the mass spectrometry) by lowering the DC offset of the array electrodes with respect to that of the cIM. However, this DC offset is still higher than the exit and postarray (after the exit). In addition, separated ions can also move

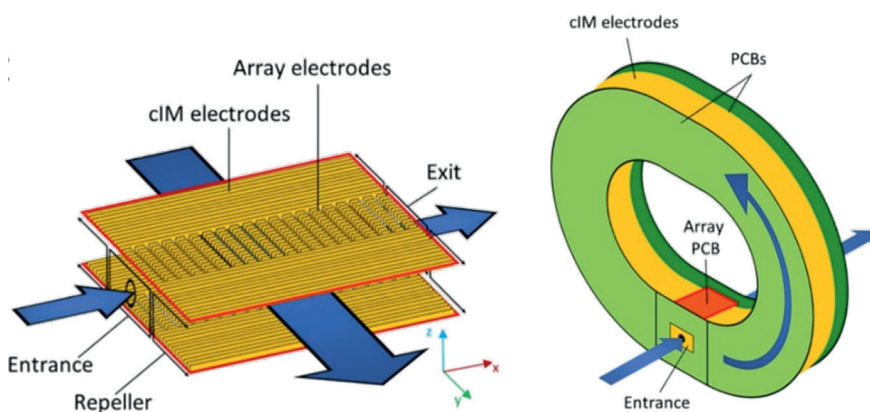


Figure 2. cIMS instrument design. (Adapted with permission from Ref. [25], Copyright (2019) American Chemical Society, Note: further permissions related to the material excerpted should be directed to the ACS).

to the prearray store if needed by adjusting the voltages accordingly. In short, this model provides a “multifunction” option for mobility selection, activation, storage, IMSⁿ, as well as custom combinations of these functions.

Interestingly, cIMS provides a resolving power of around 80 for a single pass while 750 for 100 passes, but also loses its transmission ion efficiency with each pass (~2.4% per pass) [25]. Additionally, much work on cIMS has been conducted showing promising separation results [53–55]. For example, Williamson et al. utilized mass distribution-based isotopic shift separation for the characterization of isomeric species and conformers with very good reproducibility [56]. Harrison et al. reported the use of cIMS coupled with a temperature-controlled electrospray ionization source to analyze large biomolecular systems, temperature-induced protein aggregation, and oligonucleotide complexes [55]. Another study incorporated liquid extraction surface analysis with cIMS for the analysis of intact proteins in mouse brains and rat kidneys, increasing the number of proteins being detected with each pass using multipass experiments [54].

4.2 Structures for lossless ion manipulations (SLIM)

Nearly a decade ago, SLIM platforms were developed to demonstrate their application in high-resolution IM separation. A SLIM device consists of two parallel boards which are fabricated using printed circuit board (PCB) technology, which is fast and inexpensive. SLIM boards can also be fabricated using 3D printing or other processes. Ion motion within SLIM is driven by the action of net forces caused by the electric field (DC and/or TW) and collision with a buffer gas while efficient ion confinement is achieved by applying a combination of high frequency (RF) and DC “guard” voltages. The applied fields in SLIM create “an ion-pipe” in which the ions are transmitted “losslessly” through the device (**Figure 3**). Therefore, the ions can travel different paths in the SLIM by changing or removing the electric field [57]. In addition, simulations of ion motion and the applied electric fields within the SLIM with the choice of fields RF and DC are investigated using computational methods such as SIMION [58, 59].

Different SLIM concepts are used for ion mobility separation based on the constant field SLIM and the traveling wave SLIM (TW-SLIM). In the constant field SLIM, the first generation of SLIM, constant DC fields (e.g. DTIMS) are used for ion motion and ion mobility separation with a resistor divider network to establish a voltage gradient across the electrodes and a capacitor chain for the superimposed Ref. [57]. However, the disadvantage of the constant field SLIM is the limitation of ion path

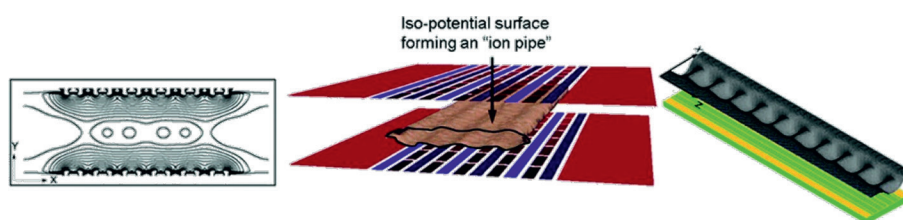


Figure 3.
The RF potentials are presented by the contour lines on the left. The central plane shows the ion confinement region (iso-potential surface forming an “ion pipe”). The right plane represents the TW potential distribution between two SLIM boards and the ion confinement region. (Adapted with permission from Ref. [57], Copyright (2017) Royal Society of Chemistry).

length and field strength because longer ion path lengths (e.g., 13 m) would require extremely high voltages. In contrast, since TW-SLIM repeatedly uses the same TW sequence across the device utilizing a low voltage (e.g., 30 V) enabling the extension of the IM path length significantly without the need for high voltages. In addition, the advantages of TW-SLIM include that it does not require many electronic components (resistors and capacitors). It uses phase-shifted RF waveforms for adjacent electrodes that are in the direction of the ion path, and segmented TW electrodes that are interleaved [60, 61]. Recently, the TW-SLIM model has been modified to a multi-level TW-SLIM where multiple dual-surface SLIM boards are stacked vertically, and each level contains “ion escalators” and “ion elevators” between the SLIM levels [62]. Moreover, a resolving power of ~400 was obtained with a multilevel ion path of only 43.2 m [63]. In addition, other parameters, such as plate spacing, thickness, velocity, and amplitudes can affect the ion transfer efficiency [2, 63]. Many SLIM studies are still being studied on improvement (i.e., the charge capacity), modification of SLIM designs, and coupling with different MS platforms [64–67].

5. Theoretical approaches for CCS

Even though ion mobility experiments are rapid, they still have some limitations, such as the CCS accuracy, which is affected by the calibrant mixtures available for CCS calibration and the calibration method. Another limitation is the ability to identify compounds in untargeted analysis when no reference CCS values are available for the specific chemical class of interest. To solve this problem, theoretical approaches such as computational and machine-learning prediction models have been used as alternatives. More details on different CCS prediction theoretical models were recently summarized by Kartowikromo et al. [51].

5.1 Computational models

Computational models have been used for decades to calculate CCS values. CCS Predictions using these models require conversion to 3-dimensional (3D) structures to obtain all possible conformers with consideration of variables such as bond length, and protonation/deprotonation sites [68, 69]. These conformers are then optimized to the minimum energy, which is used to calculate the CCS values on a computational software platform, such as IMoS [70–72], MOBCAL [73, 74], and Sigma [75]. Some of the recent computational platforms are Rosetta online server that includes everyone (ROSIE) [76], IMPACT [77], and colloidoscope [78]. The optimization of the conformers is performed using various theoretical approaches, including density functional theory (DFT), molecular dynamics, and molecular mechanics, while the calculation of CCS is performed using algorithms, such as projection approximation (PA), exact hard-sphere scattering (EHSS), and trajectory method (TM) [68]. One of the earliest CCS prediction methods in helium gas was based on the PA algorithm using the average projected area of a large number of orientations of the analyte [79]. Furthermore, other computational CCS calculation methods have been developed, showing some improvement compared to the previous methods, such as superposition approximation (PSA) [80], diffuse hard-sphere scattering (DHSS) [70], and colloidoscope TM [78]. Olajide et al. applied the IMoS TM method to structurally characterized two conformers of verapamil based on their calculated CCS [39].

Recently, a web application (<https://rosie.graylab.jhu.edu>) for CCS calculations using the projection approximation rough circular shapes (PARCS) algorithm on ROSIE was reported where the projection area of each atom with a nine points rough circle was studied. However, computational approaches usually require high CPU power (computationally intensive) to generate a whole set of compounds which can even take days even though it can accurately calculate CCS values. In addition, it is also prone to larger CCS errors, especially for flexible molecular structures [81].

5.2 Machine learning (ML) models

Machine learning-based models have the advantage of less time and CPU power for predicting multiple connections with fewer errors compared to computational methods. In fact, errors of less than 10% are obtained compared to the computational methods. These models require a training set with data obtained from experimental measurements and a validation dataset divided into an internal and an external dataset [69]. In addition, molecular descriptors (MDs) are determined for the compounds in the dataset, which are numerical values obtained from molecular structures using mathematical algorithms, such as measured values (polarity, logP, dipole moment, etc.) and theoretical values (constitution, geometry, physical chemistry, etc.). These MDs are calculated or determined using computer software or programs such as Dragon, or online databases such as Lipid Maps and the Human Metabolome Database (HMDB) [82]. The combination of different MDs can provide a “finger-print” for the chemical property of a compound. However, these MDs do not always correctly reflect the features of a compound’s structure [83]. Moreover, the MDs are preprocessed, selected, and optimized using the preferred ML algorithm. ML algorithms can be divided into nonlinear and linear methods, such as regression models, neural networks (NN), random forest (RF), or Gradient Boosting Machine (GBM) [82]. Several ML CCS prediction models that can be used for lipidomics and metabolomics studies are publicly available and are based on support vector regression such as MetCCS [84], LipidCCS [85], AllCCS [69], CCSbase [83], etc. Bijlma et al. were the first to present an ML-based model for predicting the CCS values for small molecules based on the ANN algorithm (a type of NN algorithm) [86]. Subsequently, many neural network algorithm methods and comparisons between different neural network algorithms have been reported to be used for different omics (lipidomics, metabolomics, and proteomics), in which some methods are publicly available such as DeepCCS [87], DarkChem [88], AlphaPeptDeep [89], etc. Although ML models provide fast CCS predictions with lower errors, they still have some limitations due to the availability of databases for a variety of chemical classes, the low resolving power of IMS instruments, and the calibration methods for more accurate experimental CCS values.

6. Applications of IM-MS to lipidomics, metabolomics, and proteomics

6.1 Lipidomics

Lipids are structurally diverse with various isomers performing different biological functions which has necessitated the use of IMS for their structural elucidation and isomeric separation. IM-MS separates various lipid categories, with each

category occupying a distinct m/z -drift time or (m/z -CCS) trendlines, mainly due to their unique backbones. For instance, Sphingolipids (e.g., Cer, SM, and HexCer) have more extended structures and longer drift times than glycerophospholipids (e.g., PC, PA, PE, PG, etc.) [90]. Within each category, IM-MS further separate the lipids into classes according to their headgroups. A longer fatty acids chain in a lipid class induces a more extended structure, and the high number of double bonds generates a bend and compact structure. In fact, there is a 1–5% reduction in drift time with the addition of one double bond in the acyl chain of PC, PS, PE, etc. [90]. Lipid species exist as different isomers, such as positional isomers (e.g., sn- and double-bond positional isomers), stereoisomers (e.g., cis/trans isomers and enantiomers), and acyl chain isomers. These different isomeric systems differ by less than 1% in their CCS values, requiring high-resolution IM instruments for their baseline resolution [90]. Groessl et al. utilized AP-DTIMS with an IM resolving power of ~250 to separate sn-positional isomers (PC (16:0/18:1) vs. PC (18:1/16:0)) and double-bond positional isomers (PC(18:1(9Z)/18:1(9Z)) vs. PC(18:1(6Z)/18:1(6Z)) through specific adduct formations (e.g., Ag^+) [9]. The Agilent DTIM-MS 6560 instrument was used to partially distinguish cis/trans isomeric pairs (FA(18:1(9Z)) vs. FA(18:1(9E))) while the Waters TWIM-MS enabled the separation of carotenoid cis/trans isomers, such as cis- β -carotene and all trans- β -carotene [91, 92]. Serpentine ultralong path with extended routing (SUPER) enabled baseline separation of cis/trans isomers of glycerophospholipids (e.g., PC (16:1(9Z)/16:1(9Z)) vs. PC (16:1(9E)/16:1(9E))) and partially separated the stereoisomers of sphingolipids (e.g., GlcSphingosine (d18:1) vs. Gal-Sphingosine(d18:1)) [93]. High-resolution TIMS-MS was used to successfully identify lipid isomers that differ in the double bond locations/geometries as well as in the position of the acyl chain with resolving power up to ~410 [94]. A FAIMS study showed that about 75% of triacylglycerol (TG), and PC isomers could be separated at high electric fields, including regio-, sn-, positional, and geometric pairs [95]. In theory, if an IM resolving power of 500 and above is utilized, most lipid isomers would be resolved [90]. Moreover, IM improves lipid identification accuracy through the measurement of its CCS values. Several research groups have experimentally measured lipid CCS to confidently support lipid identification. Groessl et al. [9] and May et al. [96] acquired the CCS values of 112 and 294 lipids with DTIMS, respectively covering glycerophospholipids and sphingolipids. Picache et al. developed a curated CCS database with more than 3800 CCS values using DTIMS with 810 of those CCS values being lipids [97]. CCS values of 244 lipids from 13 lipid classes were acquired with TWIMS by Paglia et al. [98]. Hines et al. developed a lipid CCS database with 148 lipids and 258 CCS values using biological samples with further expansion in their subsequent work [99]. Hernández-Mesa et al. measured 1080 CCS values from 300 steroids using TWIMS [100]. The full details of these experimental CCS measurements can be found in previous reviews [14, 90] and a summary is provided in a review by Kartowikromo et al. [51]. The limited experimental lipid CCS values compared to a large number of possible lipid structures have necessitated the development of Machine Learning models for the prediction of lipid CCS values such as LipidCCS [85].

6.2 Metabolomics and proteomics

Metabolomics is a large-scale study of metabolites, that is, small molecules (<1500 Da) involved in cell function and various regulatory pathways.

Biological processes often produce metabolite isomers during metabolism which common analytical methods often fail to distinguish. Hines et al. successfully determined the nitrogen CCS values of 1425 drug or drug-like metabolites using TWIMS, resolving drug metabolites protomers such as fluoroquinolone protomers in her study [101]. DTIMS was utilized in the isomeric separation of steroid metabolites [102], isomeric bile acid [103], and isobaric/isomeric biomarkers in newborn screening [104]. The Zheng-Jiang Zhu group collected more than 5000 empirical metabolites CCS values from literature to predict the CCS for more than 1.6 million small molecules thereby improving unknown metabolites annotation in untargeted metabolomics [105]. Multiplexing strategy in DTIMS has significantly improved sensitivity in metabolomics with applications such as the analysis of contaminants of emerging concerns (CECs) in human urine samples. This study introduces the first comprehensive database of $^{DT}CCS_{N_2}$ values of 148 CECs, and their metabolites collected utilizing the Agilent 6560 platform [106]. PNNL Preprocessor, developed by the Smith group is used for the simplification of the multiplexed data through data interpolation, demultiplexing, multidimensional smoothing, and saturation repair functions [107]. Twenty seven diverse metabolites covering a mass range of m/z 90–788 demonstrate the suitability of DTIMS in the multiplexed mode for non-targeted metabolomics in difficult matrices [108]. TIMS has also been used successfully to analyze isomeric opioid metabolites in human urine and does so with better precision and reproducibility than standard multiple reaction monitoring (MRM) techniques [109]. TIMS has been used to study human colorectal cancer utilizing metabolomics and multi-omics approaches [110]. Traditional untargeted metabolomics studies have detailed the utility of FAIMS in the separation and distinction of metabolites [10]. Metabolites isomer separation by cIMS includes separation of diastereomeric pairs of enantiomeric dimers [111], characterization of protomers of fluoroquinolone antibiotic residues [112], and separation of positional isomers of Diclofenac Acyl Glucuronide [113]. Three glycol-BA isomers (glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA), and glycoursoodeoxycholic acid (GUDCA) were separated by SLIM SUPER IM-MS as both their cyclodextrin complexes and as their potassiated adducts as shown in **Figure 4** [114]. The CCS values collected on IM-MS platforms including 400 metabolites (DTIMS) by Zhou et al., more than 500 metabolites and xenobiotics (DTIMS) by Zheng et al., 417 metabolites (DTIMS) by Nichols et al., 125 metabolites (TWIMS) by Paglia et al., and 510 metabolites (TWIMS) by Nye et al., have been detailed in a recent review [14].

Protein conformers have been studied using TIMS which was used for conformational analysis of several model peptides [115]. DTIMS was utilized to measure the gas-phase conformational populations of three proteins: ubiquitin, cytochrome c, and myoglobin reporting over 260 helium and nitrogen cross section values for the three proteins [116]. cIMS has been utilized to study the gas-phase stability of protein ions and found that the native protein conformation is stable on the order of hundreds of milliseconds [117]. The applications of DTIMS, TWIMS, FAIMS, cIMS, and TIMS for various proteomic applications have been extensively detailed in a recent review [10].

7. Conclusion

IM-MS combines structural differentiation and mass analysis, improving peak capacity, resolution, sensitivity, selectivity, and isomer separation. When coupled with chromatographic separation techniques, for instance, LC-IM-MS/MS, compound

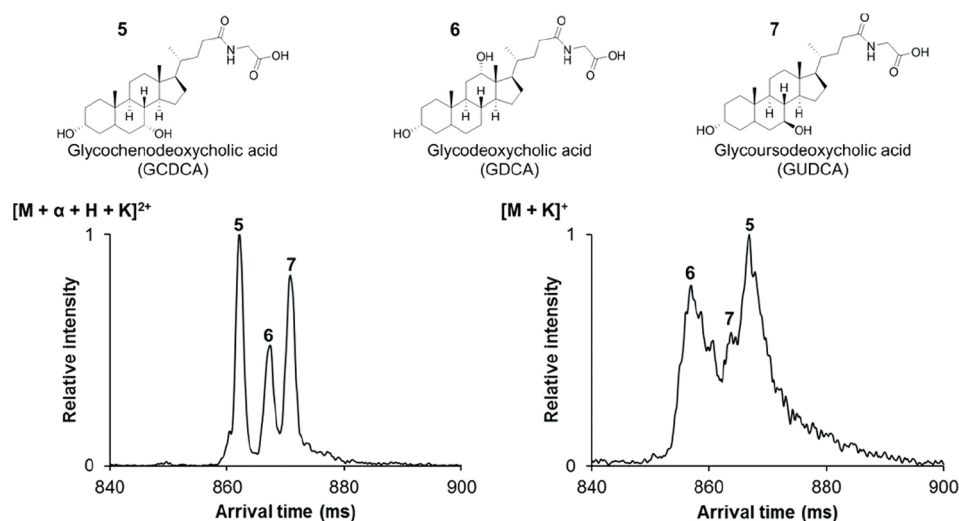


Figure 4. SLIM SUPER IM separation of three glycol-BA isomers. 72 m SLIM SUPER IM separation as $[M + \alpha + H + K]^{2+}$ ions (left) and 85.5 m SLIM SUPER IM separation as $[M + K]^+$ ions (right), where M is the BA and α is the cyclodextrin. (Adapted with permission from Ref. [114], Copyright (2018) American Chemical Society).

identification in targeted and untargeted omics analysis is improved using the four-dimensional data (i.e., RT, CCS, m/z , MS/MS). The IM separation mechanisms, resolution, duty cycle, and applications are thoroughly discussed for the comprehension of researchers in the selection of IM instruments suitable for their experiments in this chapter.

Low resolution in IM-MS instruments is the major restriction for isomer separation. For instance, the CCS difference between stereoisomers and enantiomers is less than 1% and 0.1% respectively. IM-MS instruments like DTIMS and TWIMS have resolutions of 40–60 and can only distinguish isomers with CCS differences in the range of 1.5–3%. The development of high-resolution IM-MS instruments including SLIM and cIMS with resolving power above 400 has made it possible to separate isomers with CCS difference less than 1% and can improve the development of portable ion mobility devices, especially for on-site analysis. Experimental CCS values are needed by machine learning models to accurately predict CCS; hence the use of high-resolution IM instruments would improve the accuracy of CCS prediction since challenging isomers would be separated and their CCS would be measured accordingly. This would greatly promote the application of IM-MS in the identification of unknown compounds. Improvements in IMS resolution, standardization of instrument calibration (preferred calibrant ions, etc.), enhancement of CCS database, development of CCS processing software, and coupling with other separation and MS analysis strategies are all important factors that continuously need improvement for the advancement of IM-MS and applications in all-omics studies.

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