



HAL
open science

Ion Mobility -Mass Spectrometry: An Overview

Valérie Gabelica

► **To cite this version:**

Valérie Gabelica. Ion Mobility -Mass Spectrometry: An Overview. Frank Sobott; Alison Ashcroft. Ion Mobility-Mass Spectrometry: Fundamentals and Applications, Royal Society of Chemistry, pp.1-25, 2021, New Developments in Mass Spectrometry, 978-1-83916-166-7. 10.1039/9781839162886-00001 . hal-03463389

HAL Id: hal-03463389

<https://hal.science/hal-03463389>

Submitted on 2 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Ion Mobility-Mass Spectrometry: An Overview

Valérie Gabelica

Univ. Bordeaux, CNRS, INSERM, ARNA, UMR 5320, U1212, IECB, F-33600 Bordeaux, France

v.gabelica@iecb.u-bordeaux.fr

Abstract

Ion mobility spectrometry is increasingly often coupled to mass spectrometry measurements, either for separation purposes or to assist compound identification. This chapter introduces basic definitions and concepts underlying ion mobility spectrometry. The definition of “collision cross-sections” as used in ion mobility spectrometry is also discussed, with a cautious note that the IUPAC definition is not entirely suited to describe the physical quantity on which ion mobility depends. Finally, the types of ion mobility analyzers most commonly encountered in contemporary commercial ion mobility - mass spectrometers are introduced and compared.

1. What is ion mobility spectrometry?

1.1. Spectrometry

A *spectrometric* technique physically separates compounds in a so-called spectrometer. A *spectroscopic* technique, in contrast, analyses the interaction between matter and electromagnetic radiation (UV, visible, infrared light, etc.).

The most widespread spectrometric technique is *mass spectrometry*, which physically separates compounds according to their mass-to-charge ratio. In practice, mass spectrometry separates ions, not neutral compounds, because the separation is achieved by the movement of ions in an electric or magnetic field. To ensure that the ion movement is defined only by the electric or magnetic field, as desired in most mass analysis approaches, mass spectrometers operate at low pressure so that collisions do not interfere with the movement of the ions during mass analysis.

1.2 Ion Mobility

Imagine you want to move ions using an electric field (\vec{E}). The electric force applied to the ions is $\vec{F} = q\vec{E}$. The higher the charge q , the higher the force \vec{F} . *Because of the electric field, the ions will accelerate*, according to the law $\vec{F} = m\vec{a}$, where \vec{a} is the acceleration.

Now let us imagine that the ions are accelerated in a medium filled with gas, at a high enough gas pressure that there are many collisions to compensate for the acceleration. *Because of the collisions, the ions will slow down*. The collisions are responsible for a friction force, acting in the opposite direction to the applied electric force. So, when ions are subjected to an electric field in a region of relatively high pressure, they will constantly be accelerated, decelerated, accelerated, decelerated, and so on. (Figure 1).

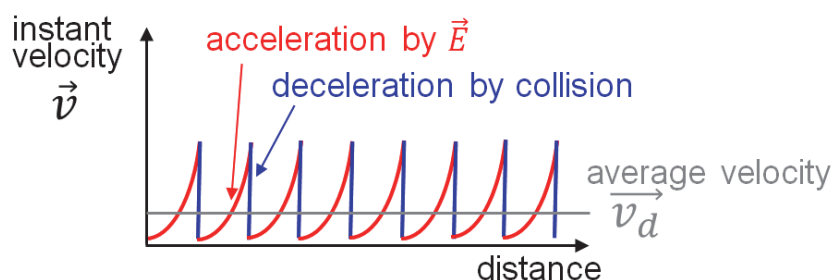


Figure 1: The instant velocity of an ion is constantly changing, and the average drift velocity \vec{v}_d depends on the balance between accelerations by the electric field \vec{E} and decelerations by collisions.

If the collisions are frequent and numerous enough, the electric force and friction force balance each other and a *stationary state* is reached. As the two forces cancel each other out, there is no net acceleration, and the average speed will appear constant. This is called *drift velocity* (\vec{v}_d).

The ion's mobility (K) is the proportionality constant between the drift velocity and the electric field:

$$\vec{v}_d = K \cdot \vec{E} \quad (1)$$

In summary, ion mobility spectrometry consists of separating ions in an electric field in the presence of a collision gas. The separation will be based on the value of K , the ion's mobility. This chapter will cover the very basics of ion mobility spectrometry. For a thorough coverage of ion mobility theory, the reader can refer to a recent book by Larry A. Viehland.¹

2. What is ion mobility spectrometry used for?

The mobility, K , of an ion depends on its charge q ($q = ze$, where z is the net charge and e the charge of an electron), and its friction in the gas. We are interested in measuring this friction. Indeed, even if friction is partly related to mass (ions of higher mass are usually larger as well), other parameters come into play, for example the arrangement of atoms in space (the three-dimensional structure) of the ion.

At equal mass and charge, if an ion has a more expanded structure, the friction will be greater, thus the mobility will be smaller, and thus the drift velocity will be lower. If the ion has a more compact structure, its mobility will be larger, resulting in a higher drift velocity.

This is the "parachute" effect. If you jump out of an airplane, you are subjected to the force of gravity. But you can slow down your fall by deploying your parachute. Your mass does not change, the force of gravity does not change, but your "conformation" changes and slows you down. The larger your parachute, the slower your fall (Figure 2).



Figure 2: An ion mobility analogy: the larger your parachute, the slower your fall.

Ion mobility spectrometry separates ions according to their three-dimensional structure (shape, i.e. nuclei positions but also, as we will see below, electronic structure), and thus provides complementary information to mass spectrometry. Ion mobility spectrometry is particularly useful for separating isomeric compounds (which have the same atoms but different three-dimensional arrangements), or isobaric compounds (which incidentally have the same mass). Since both spectrometries are performed on ions in the gas-phase, they are frequently coupled into a single instrument: an ion mobility - mass spectrometer (IM-MS). Consequently, ion mobility spectrometry can be used for many different applications, as detailed below.

2.1. An additional method of separation coupled to mass spectrometry.

The ion mobility separation typically takes place on the millisecond time scale, i.e. orders of magnitude faster than chromatography, and thus the methods can be used orthogonally. The IM-MS combination is particularly useful:

- **To resolve conformational isomers.** Figure 3 shows an example with the separation of two conformations for the dinucleotide dCG (deoxycytosine-deoxyguanine).² Here the mobility separation was carried out in a temperature-controlled drift tube. Folded conformations travel faster (arrive earlier) than open conformations, but it is interesting to note that the two peaks are separated only at low drift tube temperature. This illustrates that one condition for separating conformers by ion mobility spectrometry is that these conformers should not inter-convert in the gas phase on the time scale of the experiment. At room temperature (300K), only one peak appears, with an arrival time mid-way between the arrival times of the two conformers because every molecule

spends a fraction of its drift time in the folded conformation and another fraction of its drift time in the unfolded conformation.

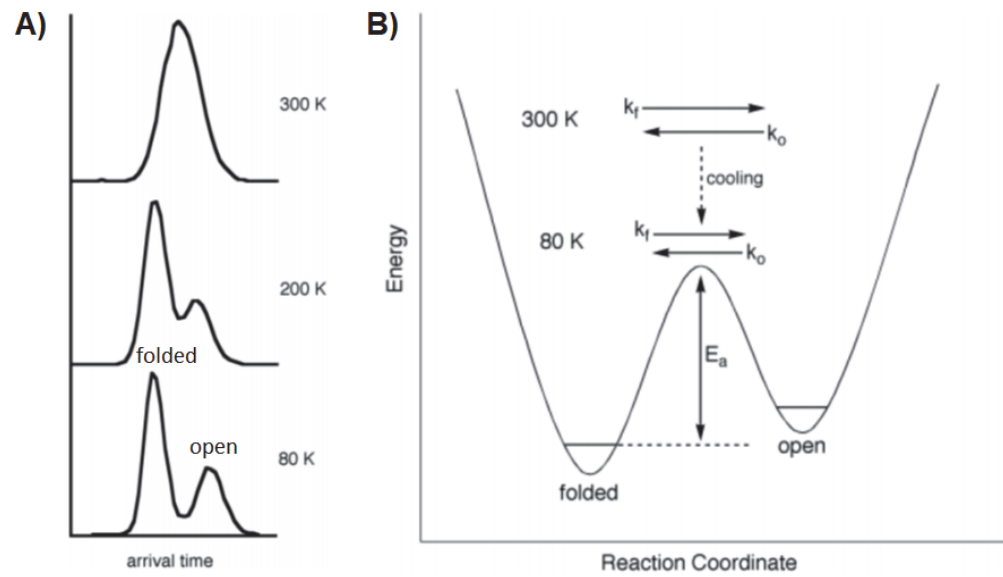


Figure 3: A) Arrival time distributions for the dinucleotide dGC measured at different temperatures. B) Simple reaction coordinate diagram explaining the isomerization of the two conformers. Adapted from reference ² with permission from Springer Nature, Copyright 2002.

- More generally, **for separating complex mixtures**, given the high probability of encountering isobars. A historical example is shown in Figure 4, wherein carbohydrates, peptides and lipids can be separated based on their mass/drift time trends.³ Detecting signals on a different mass/mobility trend line can help discover new components in the mixture.

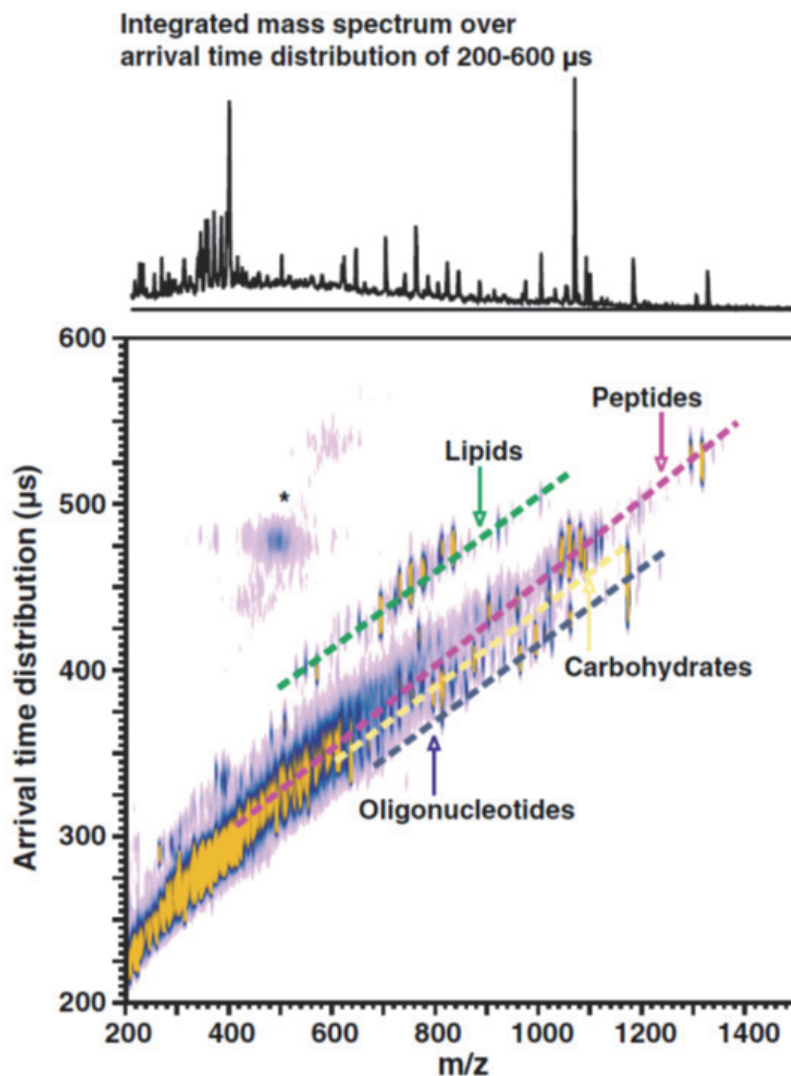


Figure 4: A plot of MALDI-IM-MS conformation space obtained for a mixture of model species representing each molecular class as labelled. The mass separation is horizontal and the mobility separation (arrival time distribution) is vertical. The dashed lines are for visualization purposes. Adapted from reference ³ with permission from Springer Nature, Copyright 2009 Springer-Verlag.

- **As a filter to extract specific, targeted signals.** Figure 5 shows the extraction of DNA signal from a salty sample.

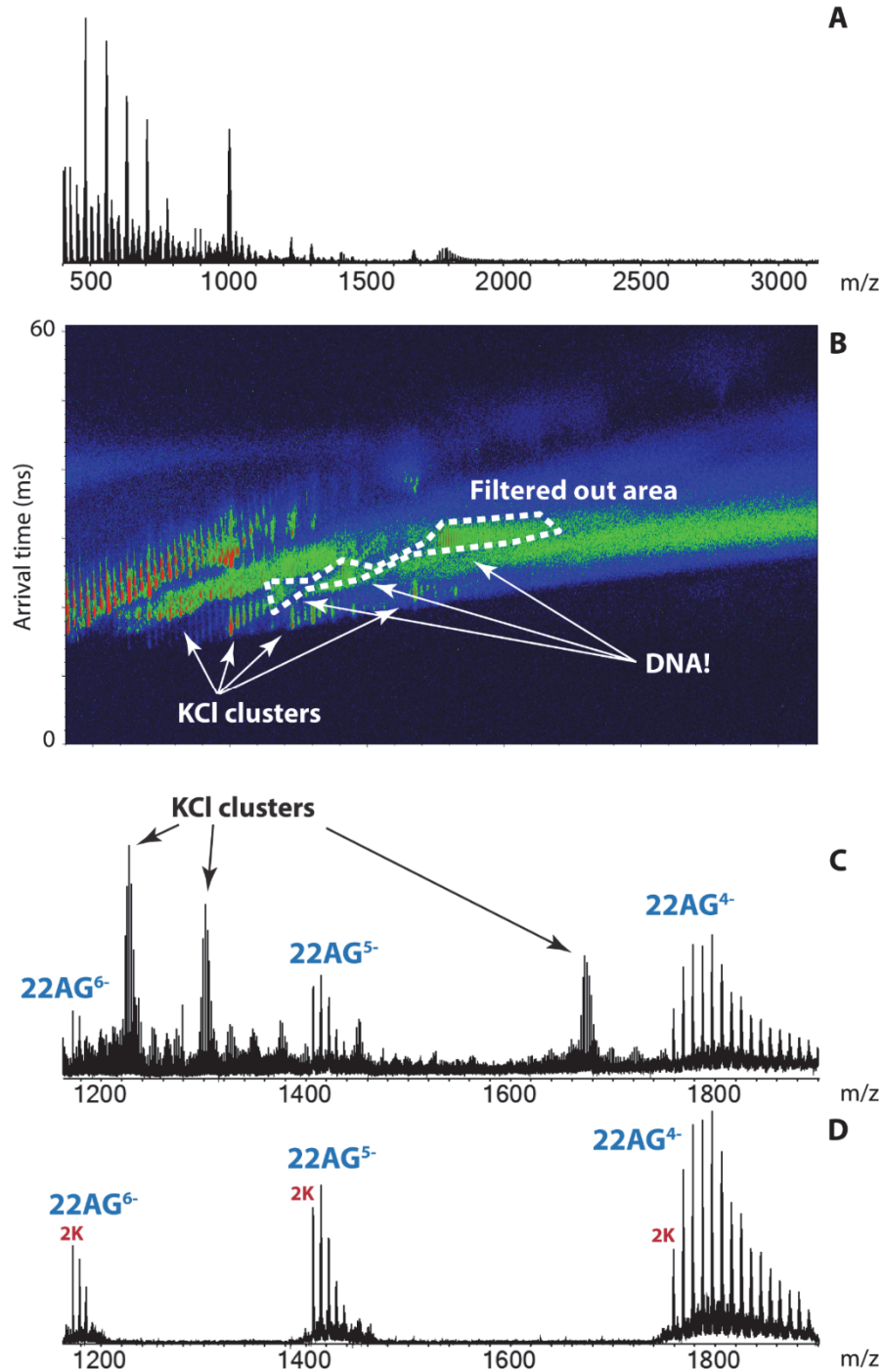


Figure 5: Mass spectra of 10 μM oligonucleotide $\text{d}(\text{A}(\text{GGGTTA})_3\text{GGG})$ in 100 mM trimethylammonium acetate + 10 mM KCl. A) Full scale mass spectrum, B) the 2D map of the arrival time (mobility dimension) as a function of the m/z , C) zoom on the m/z region of interest for DNA in the mass spectrum and D) the mass spectrum reconstructed from selecting the area indicated in B.⁴

- A more mass spectrometry-specific use of ion mobility is **the separation between charge states**. This is particularly useful for interpreting electrospray ionization mass spectra, where several mass and charge values can result in a given m/z ratio. For example, a dimer with twice the mass and twice the charge of a monomer will have the same m/z ratio as the monomer. However, such a dimer usually drifts faster than the monomer, because the electric force is twice the one applied to the monomer ($\vec{F} = ze\vec{E}$), but the friction is usually less than twice that of the monomer. Figure 6 shows an example with the separation of a peptide monomer²⁺, dimer⁴⁺, trimer⁶⁺ and tetramer⁸⁺.⁵

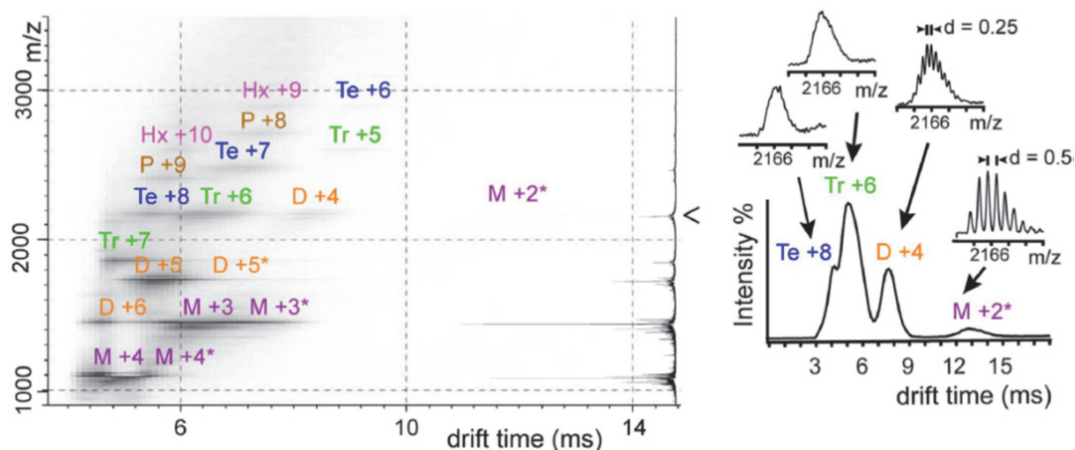


Figure 6: ESI-IM-MS separation of a low-molecular weight fraction of the amyloid beta peptide A β 40. The mass dimension is vertical, the mobility dimension (drift time) is horizontal. M = monomer, D = dimer, Tr = trimer, Te = tetramer, P = pentamer, Hx = hexamer. On the right, extraction of the drift time distribution corresponding to the m/z range [2164,2169] indicated by the arrow on the right-hand side. The insets show the mass spectra extracted for each drift time portion, wherein the spacing between isotopic peaks confirms the charge states of the monomer (+2) and dimer (+4). Adapted from reference ⁵, <https://doi.org/10.1038/srep14809>, under the terms of the CC BY 4.0 license, <http://creativecommons.org/licenses/by/4.0/>.

2.2. To identify compounds based on their ion mobility

This can be carried out generally in one of two ways:

- Comparing the measured ion mobility (or any experimental value proportional to the ion mobility) to databases. These databases are generally experimental, but databases of theoretical values are also being developed using artificial intelligence.
- Converting the ion mobility into a physical quantity called collision cross-section (CCS, defined below), and confront this experimental CCS value with calculated CCS values for candidate ion structures. The structural interpretation is based on the agreement between experimental and theoretical CCS values.

Ion mobility is becoming a highly desirable feature for all high-end mass spectrometers, whether for the separation of complex mixtures, the identification of known compounds (present in a database) or for the structural characterization of unknown compounds.

3. Key definitions and equations for ion mobility spectrometry

Metrology is the science of measurement.^{6,7} A first principle of metrology is that before a quantitative value can be obtained, it is necessary to define precisely *what is being measured*. A second principle is that all measurements must be traceable to the definitions of the International System of Units (SI). A thorough discussion of the metrology of ion mobility spectrometry has been published recently, leading to recommendations to report ion mobility - mass spectrometry measurements.⁸ Measurements of an ion's mobility K are easily traceable to the SI, while the experimental collision cross-section (CCS) is a quantity derived from K , but is not itself directly traceable to its derived SI unit (i.e. here the square meter).

3.1. What is being measured: the mobility of an ion in a gas

Here we discuss that, for an analyte, the measured ion mobility value will depend (1) on the type of ion produced (type of adduct, history of formation and activation before IM analysis) and (2) on the nature and temperature of the gas. Several ion mobility values can therefore be associated with a single analyte.

3.1.1. Influence of the nature of the ion produced from the analyte

Analytes can be neutral or ionic. The ion mobility of an analyte is a gas-phase property of an ion produced from this analyte via an ionization source. Thus, one key element defining what is being measured is the way in which analytes are vaporized, desolvated, ionized, and treated after ionization. The type of ionization source, the polarity of the ions (positive or negative) will be important, as well as the type of adduct, the site of adduction, and the conformation of the ion.

3.1.1.1. Effect of polarity and type of adduct

"Type of adduct" means protonation, deprotonation, addition of cation or anion, etc. Therefore, we do not define "the ion mobility of molecule X", but more precisely, for example, "the mobility of the $[M+H]^+$ ion produced by electrospray ionization of molecule X". In this respect, the property "ion mobility" is different from mass: an analyte has only one mass, but can have several ion mobilities, depending on the type of ion produced in the source.

Moreover, the ion mobility depends on the three-dimensional structure of the ion (and more precisely, on the electronic structure resulting from the positions of the atoms). Therefore, in addition to the type of adduct (which is related to an m/z ratio), any experimental parameter that can influence the structure of the adduct is important in ion mobility spectrometry.

3.1.1.2. Effect of protonation/deprotonation/adduction isomerism

For the same total charge z , it is possible to place the charge(s) in different ways. For example, the site of protonation can influence ion mobility, and the same analyte can therefore be ionized in positive mode into several kinds of $[M+H]^+$ ions. An example is shown in Figure 7, with the drift tube ion mobility of benzocaine $[M+H]^+$ in nitrogen, displaying two peaks corresponding to protonation isomers.⁹ Nitrogen is a polarizable gas, and its interaction with the ion is more influenced by the electronic distribution around the nuclei, explaining the higher friction (longer drift time) with the protomer having the highest dipole moment created by the partial charges (blue/red coloring of the atoms). Experimental parameters that can influence the protonation/deprotonation/adduction sites are: the solvent, the vapors present in the source, and the pressures and voltages in the source.

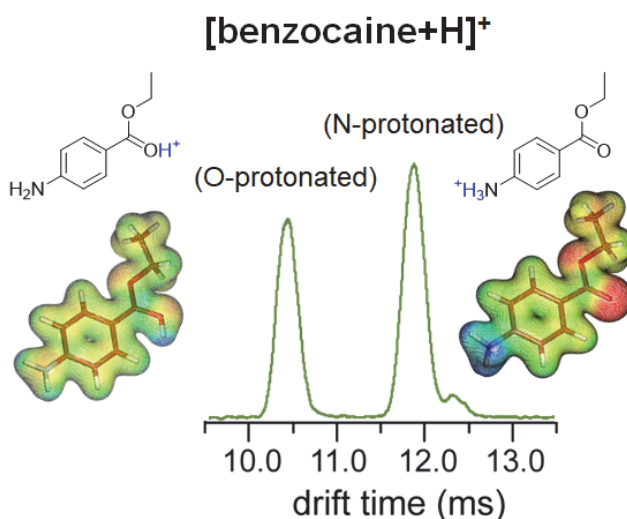


Figure 7: Effect of the protonation isomer on the drift ion mobility of benzocaine. The shape is not affected by the proton location, but the charge distribution (indicated by blue/red colors) is very different. The N-protonated isomer has a larger dipole moment. Data and structures courtesy of K. Pagel.

3.1.1.3. Effect of conformation

Finally, even if the positions of the adducts (protons or counterions) are equal, the three-dimensional conformation may vary. Following ionization, the conformation obtained is not necessarily the most stable in the gas-phase. Conformations close to those in solution can initially be formed due to the presence of non-covalent bonds, but if more energy or time is given to the ions, the more likely they are to overcome rearrangement barriers, to attain the most stable conformation in the gas phase. Note also that a population of ions can have a distribution of conformations.

Preserving in the gas-phase as many as possible of the non-covalent interactions that were present in solution is particularly interesting if one wants to deduce the conformations of the analytes in solution thanks to ion mobility spectrometry. The experimental parameters that will influence the conformation in the gas-phase are: the solvents, co-solvents and electrolytes present in the sample (everything that

influences the electrospray process itself), the voltages and pressures in the source and the transfer zone of the ions to the ion mobility cell (everything that influences the internal energy of the ions), and the time spent at each stage.

3.1.2. Influence of the nature of the gas and of its temperature.

Ion mobility is a property related to the friction with a gas. The nature of the gas thus influences this friction. First of all, gas molecules have different sizes, and the larger the gas molecule, the greater the probability of collision and the greater the friction. In addition to the size of the gas, we must also take into account its polarizability. To grasp this aspect, we should not imagine gases and ions as hard spheres. Atoms are made of nuclei, electrons, and a lot of void. Thus, atoms are far from being hard spheres.¹⁰ Electronic clouds are "polarizable", meaning that they can deform near other charges. If a gas is more polarizable, it means it will feel from further away that it is approaching other charges, causing greater apparent friction.

In addition, the range of interaction between the ion and the gas molecules depends on their relative speed. This average relative speed depends on the temperature (see 3.3.2.2). At lower temperature (slower encounters), the gas has time to perceive the charges of the ion from further afar. Conversely, at high temperature, the gas and the ion pass by each other more quickly, have less time to interact, and therefore need to be closer to each other to interact. Beware, this is not the only way in which temperature influences ionic mobility; see 3.3.2.2.

In summary, the "collision radius" of a gas is a relative quantity which depends on its nature (size, polarizability) and its temperature. We cannot define the "mobility of an ion" in isolation, but we must always define in which gas (or mixture of gases) the ion mobility is measured and at what temperature.

3.2. Units of ion mobility

Let us start again from equation (1) defining ion mobility, and isolate K :

$$K = \frac{v_d}{E} \tag{2}$$

The electric field E is equal to the electric potential difference between two points (ΔV) divided by the distance between these points (L , for length). The drift velocity v_d is the distance travelled (L) divided by the travel time (t_d , for drift time). We have:

$$K = \frac{\frac{L}{t_d}}{\frac{\Delta V}{L}} = \frac{L^2}{t_d \Delta V} \tag{3}$$

Using SI units, L is expressed in meters, and t_d in seconds. ΔV is expressed in Volts (V), a unit derived directly from the SI.¹¹ The SI unit of K is therefore $\text{m}^2 \text{V}^{-1} \text{s}^{-1}$, but for practical reasons of orders of magnitude, the ion mobility K is usually expressed in $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$ ($= 10^{-4} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$).

3.3. Reduced ion mobility (K_0)

3.3.1. Linear correction of K for its collision frequency dependence

Returning to the concept of Figure 1, the average drift velocity depends on the balance between acceleration and deceleration. Deceleration occurs with each collision. The frequency of collisions will therefore influence the average velocity at a given field, and thus influence the ion's mobility.

The collision frequency depends on the gas number density (N = number of gas molecules per unit volume; unit: m^{-3}). For a given electric field, when N increases, the collision frequency increases and the ions have less time to accelerate between two collisions, therefore the drift velocity decreases, and consequently the mobility K decreases.

N is related to the temperature and pressure of the gas. If we take the relation of perfect gases ($pV = nRT$), where p is the gas pressure, V is the volume, n is the number of moles of gas, R is the constant of the perfect gases and T is the temperature, then:

$$N = \frac{n \cdot N_A}{V} = \frac{p \cdot N_A}{RT} = \frac{p}{k_B T} \quad (4)$$

N_A is the Avogadro constant ($6.022\,140\,76 \times 10^{23} \text{ mol}^{-1}$), and k_B is the Boltzmann constant ($1.380\,649 \times 10^{-23} \text{ J/K}$).

As the values of ion mobility K depend on N , and thus on p and T , it is not convenient to compare the values of K coming from different laboratories or even different series of measurements, as p and T may vary. In addition, it is desirable to deduce from the ion mobility experiments an intrinsic property of the ion in the gas at a given temperature (section 3.1), which is as far as possible independent of other measurement conditions. For this reason, the reduced mobility K_0 is defined as follows:

$$K_0 = K \cdot \frac{N}{N_0} = K \cdot \frac{p}{p_0} \cdot \frac{T_0}{T} \quad (5)$$

With convention $p_0 = 1 \text{ atm}$ ($101\,325 \text{ Pa}$, i.e. 760 Torr), $T_0 = 273.15 \text{ K}$, and thus $N_0 = 2.686\,7811 \times 10^{25} \text{ m}^{-3}$.

Caution: 1) The standard pressure traditionally used in ion mobility spectrometry is not equal to the standard pressure recommended by IUPAC (which is 10^5 Pa). For this reason, publications and research reports should always explicitly mention the values of p_0 and T_0 used. 2) This is not the only dependency of K on N . 3) This linear correction is valid in a pressure regime where the ion collides with one gas molecule at a time. This assumption may no longer be valid for very large ions at high pressure.

In summary, expressing the results in reduced ion mobility K_0 corrects for the effects of the gas number density on friction. However, this does not mean that K_0 no longer depends on pressure or temperature. We have seen in section 3.1.2. that K_0 depends on the relative ion/gas velocity, and therefore also on the temperature. Below is one further dependency of K_0 on the gas number density.

3.3.2. The reduced mobility K_0 depends on the E/N ratio: the "high field" limit

When the ions have little time to accelerate between collisions, the linear correction of **Eq. (5)** is sufficient to make K_0 independent of N . However, if the accelerations (related to E) become too strong and the deceleration events (related to N) are no longer sufficient to counteract these accelerations, then the linear correction is no longer sufficient, and K_0 starts to depend on the E/N ratio, as follows:

$$K_0\left(\frac{E}{N}\right) = K_0(\mathbf{0}) \left[\mathbf{1} + \alpha_2 \left(\frac{E}{N}\right)^2 + \alpha_4 \left(\frac{E}{N}\right)^4 + \dots \right] \quad (6)$$

The values and signs of the coefficients $\alpha_2, \alpha_4, \dots$ are difficult to predict. They also depend on the nature of the ion-gas pair.

One can exploit the fact that the reduced mobility K_0 differs at low and high E/N for separation purposes. The technique that uses this feature is called FAIMS (Field-asymmetric waveform Ion Mobility Spectrometry), DIMS (Differential Ion Mobility Spectrometry), or DMS (Differential Mobility Spectrometry). However, to this day one cannot deduce effective cross-sections of high-field collisions, and therefore we cannot make a priori interpretations of the ion structure from high-field measurements. An excellent book on nonlinear ion transport and fundamentals of FAIMS was published in 2009.¹²

Most other ion mobility techniques determine the low-field reduced mobility $K_0(0)$, although it is common to just write “ K_0 ”.

3.3.2.1. When do “high field” effects (non-linear effects) start to be detectable?

The high field effect depends on the relative orders of magnitude of the drift velocity $\overline{v_d}$ (relative velocity of ion-gas collisions in the direction of and due to the electric field) with respect to the mean thermal agitation velocity $|v_T|$ (relative mean velocity of ion-gas collisions due to random motion—or Brownian motion—at a given temperature), given by :

$$v_T = \sqrt{\frac{8k_B T}{\pi\mu}} \quad (7)$$

With k_B the Boltzmann constant, T the gas temperature, and μ the reduced mass of the ion-gas couple (ion of mass m_i and gas of mass m_g):

$$\mu = \frac{m_i m_g}{m_i + m_g} \quad (8)$$

“High field” means an E/N ratio giving non-linear effects, or in other words, an E/N ratio too large for $|v_d|$ to be negligible compared to $|v_T|$, as shown in Figure 8.

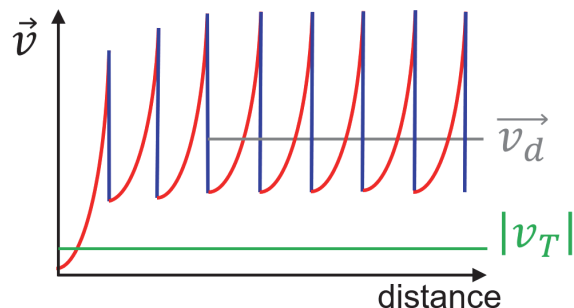


Figure 8: Instant velocity (red/blue) and average drift velocity \vec{v}_d (grey), compared to the magnitude of the thermal velocity $|v_T|$ in “high field” ion mobility conditions.

3.3.2.2. Typical field (E/N) regimes and velocities in ion mobility spectrometry

Table 1 gives orders of magnitude of thermal velocity of analyte ions of different masses, in helium and nitrogen. It depends little on the mass of the ion (except for very small ions, i.e. when m_i becomes close to m_g), but depends strongly on the nature of the gas and on the gas temperature.

Table 1: Thermal velocity v_T as a function of the nature of the gas, the mass of the ion m_i , and the temperature of the gas, according to **Eq. (7)**.

Gas	m_g (Da)	m_i (Da)	T (K)	v_T (m s ⁻¹)
He	4	200	300	1239
He	4	2000	300	1228
He	4	20 000	300	1227
He	4	200 000	300	1227
N ₂	28	200	300	495
N ₂	28	2000	300	467
N ₂	28	20 000	300	464
N ₂	28	200 000	300	464
N ₂	28	2000	100	270
N ₂	28	2000	200	381
N ₂	28	2000	300	467
N ₂	28	2000	400	539
N ₂	28	2000	500	603

To keep to simple situations with only linear effects, the E/N ratio must be such that v_D remains well below v_T . What order of magnitude of field and pressure does this correspond to? Coupling equations (1) and (5) gives:

$$v_d = K_0 N_0 \left(\frac{E}{N} \right) \quad (9)$$

Knowing that the reduced mobilities K_0 are of the order of $1 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ (0.3 to 3 in general), $N_0 = 2.686 \times 10^{25} \text{ m}^{-3}$, this gives the values of Table 2. The E/N ratio is more conveniently expressed in Townsend ($1 \text{ Td} = 10^{-21} \text{ V m}^2$).

Table 2: E/N ratio (in SI units and in Townsend; $1 \text{ Td} = 10^{-21} \text{ V m}^2$) corresponding to given drift velocities v_D , if $K_0 = 1 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$.

v_d (m/s)	E/N (V m ²)	E/N (Td)
1	1.50E-22	0.15
5	7.48E-22	0.75
10	1.50E-21	1.50
50	7.48E-21	7.5
100	1.50E-20	15
500	7.48E-20	75
1000	1.50E-19	150

In conclusion, at a temperature of 300 K, in order to have v_D 10 times smaller than v_T , one must have an E/N less than $\sim 15 \text{ Td}$ in helium, and less than $\sim 7.5 \text{ Td}$ in nitrogen. Equation (4) allows one to calculate E/N for different values of E , p and T , and obtain the orders of magnitude from Table 3.

Table 3: Typical E/N values as a function of E and p ($T = 300 \text{ K}$).

E (V/cm)	p (Torr)	E/N (Td)
1	0.5	6.21
10	0.5	62.1
100	0.5	621
1	5	0.621
10	5	6.21
100	5	62.1
10	760	0.041
100	760	0.409
1000	760	4.09

3.4. Collision cross-sections

3.4.1. IUPAC definition (σ)

The IUPAC Gold Book gives the following definition: “Collision cross-section (σ): In simple hard sphere collision theory, the area of the circle with radius equal to the collision diameter”, the latter being “the sum of radii of two colliding spherical particles”.¹³

The IUPAC definition is appropriate for “all-or-none” events. For example, techniques wherein each collision results in 100% efficient ion scattering away from its initial trajectory can directly probe collision cross-sections in the IUPAC sense (σ). This is the case of CRAFTI¹⁴ (cross-sectional areas by Fourier transform ion cyclotron resonance), frequency shifts in charge detection mass spectrometry,¹⁵ or ion dephasing in orbitraps.¹⁶

However, the IUPAC definition is problematic for ion mobility spectrometry measurements described herein, as explained in the next paragraph. Nevertheless, this was not realized in the early days of ion mobility spectrometry, and the term “collision cross-section” stayed.

3.4.2. Momentum transfer collision integral (Ω)

3.4.2.1. Why “momentum transfer” instead of “collision”?

To calculate how a collision affects mobility, it is not enough to know whether or not a collision took place, or to count the collisions. What matters is the extent to which collisions change the ion velocity. The translational momentum (or simply, “momentum”) p is related to the velocity ($p = mv$). In a collision, the total momentum ($p_{ion} + p_{gas}$) is conserved. Hence in ion mobility spectrometry, what matters during a collision is the *momentum transfer* between the ion and the gas.

3.4.2.2. Why “collision integral” instead of “cross-section”?

The term “cross-section” is a generic term in physics that describes the probability of two particles to interact, and refers to the surface area across their relative motions as they interact. For a collision by direct contact between two hard spheres i and g of radii r_i and r_g (Figure 9), along the axis of relative motion there will be a collision if the centers of mass of g and i are in a surface $\sigma = \pi(r_i + r_g)^2$.

The problem is that atoms and molecules are not hard spheres, but consist of nuclei, electrons, and void. As electron density is defined by probabilities, atoms and molecules do not actually have a well-defined boundary, and one must consider that there is a probability of interaction depending on distance. For atoms and molecules, it is therefore difficult to define σ .

Moreover, when two particles approach each other, their electron densities influence each other. This is called polarizability: electrons will tend to move when they approach a charge or a dipole. Polarizability also depends on the relative velocities of the particles: the slower the approach, the more the electrons will “feel” the other particle from a distance, and therefore the greater the polarizability. In physics, an effective cross-section is defined at a given collision energy. However, the measurement of ion mobility is done with a certain collision energy distribution, and is therefore the integral of the collision cross-sections on the collision energy distribution.

In conclusion, even if it is accepted to continue using the term “collision cross-section”, to understand the meaning of this quantity it is helpful to think in terms of the integral of momentum transfer over all collisions.

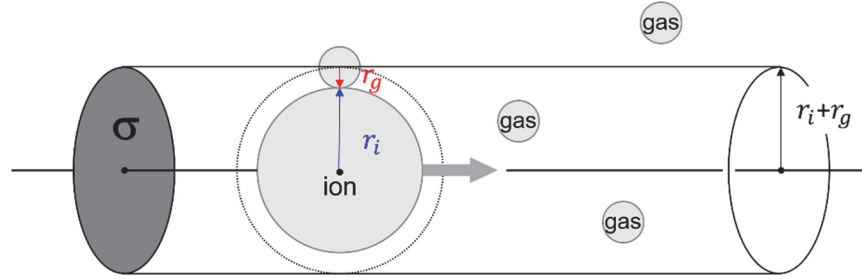


Figure 9: Effective section of collision between two hard spheres of radii r_i and r_g . Illustration by a collision cylinder of an ion moving to the right in a gas.

3.4.3. Relationship between ion mobility and collision cross-section

The foundations of the kinetic theory of ion mobility were laid by Langevin in 1905.¹⁷ The kinetic theory links K (ion mobility) to the physics of ion-gas collisions. In its contemporary form, the most commonly used equation is the following:

$$K = \frac{3}{16} \sqrt{\frac{2\pi}{\mu k_B T}} \frac{ze}{N\Omega} \quad (10a)$$

$$K_0 = \frac{3}{16} \sqrt{\frac{2\pi}{\mu k_B T}} \frac{ze}{N_0\Omega} \quad (10b)$$

μ is defined in Eq. (8). An approximation underlying Eq. (10a) is that the ion is not directionally accelerated in the gas, i.e. the electric field is zero. In practice, a validity condition for Eq. (10) is to be within the “low field limit” described above (and the full notation for the mobility should be $K_0(0)$ or $K(0)$). Equations (10) should be referred to as the “fundamental low field mobility equation”. The term “Mason-Schamp Equation” is also often used in the literature; yet this terminology is debated.¹⁸

3.4.4. Experimentally-derived collision cross-section (CCS)

From a metrological point of view, K is measured and then the collision cross-section is determined by mathematical relationships such as (10), but strictly speaking one does not measure collision cross-sections. In the combined uncertainty on the value of an experimentally-derived collision cross-section, there is therefore a contribution from the uncertainty in the mathematical relationships (10) itself, and in particular in its applicability to non-zero electric fields.

A convenient definition of the experimentally-derived collision cross-section is the value determined from a mobility measurement using Equations (10). Let us remember here that:

- Collision cross-sections have the dimensions of a surface, so its SI unit is m^2 , but it is more convenient for molecular ions to be expressed in \AA^2 (or sometimes nm^2).
- The physically rigorous name of the collision cross-section is the "momentum transfer collision integral" (symbol Ω), but nowadays we more commonly find the acronym *CCS* instead of Ω .

- Its derivation from mobility measurements using Eqs. (10) is straightforward and experimental Ω or CCS values are indicative of the “apparent size” of an ion in a gas. Yet their prediction from ion and gas properties is not straightforward.
- An ensemble of ions can be polymorphic, and thus have a distribution of collision cross-sections and not a single CCS value.¹⁹

4. How is ion mobility measured?

Figure 10 summarizes the working principles for the most widespread ion mobility devices. Ion mobility measures how ions move in a gas under the influence of an electric field. As ΔV , L and t_d are more directly measurable than E and v_d , Eq. (3) defines the practical realization of the measurement of the ion mobility K . If the potential is known at any point in the device, and the travel time is measured, K can be determined.

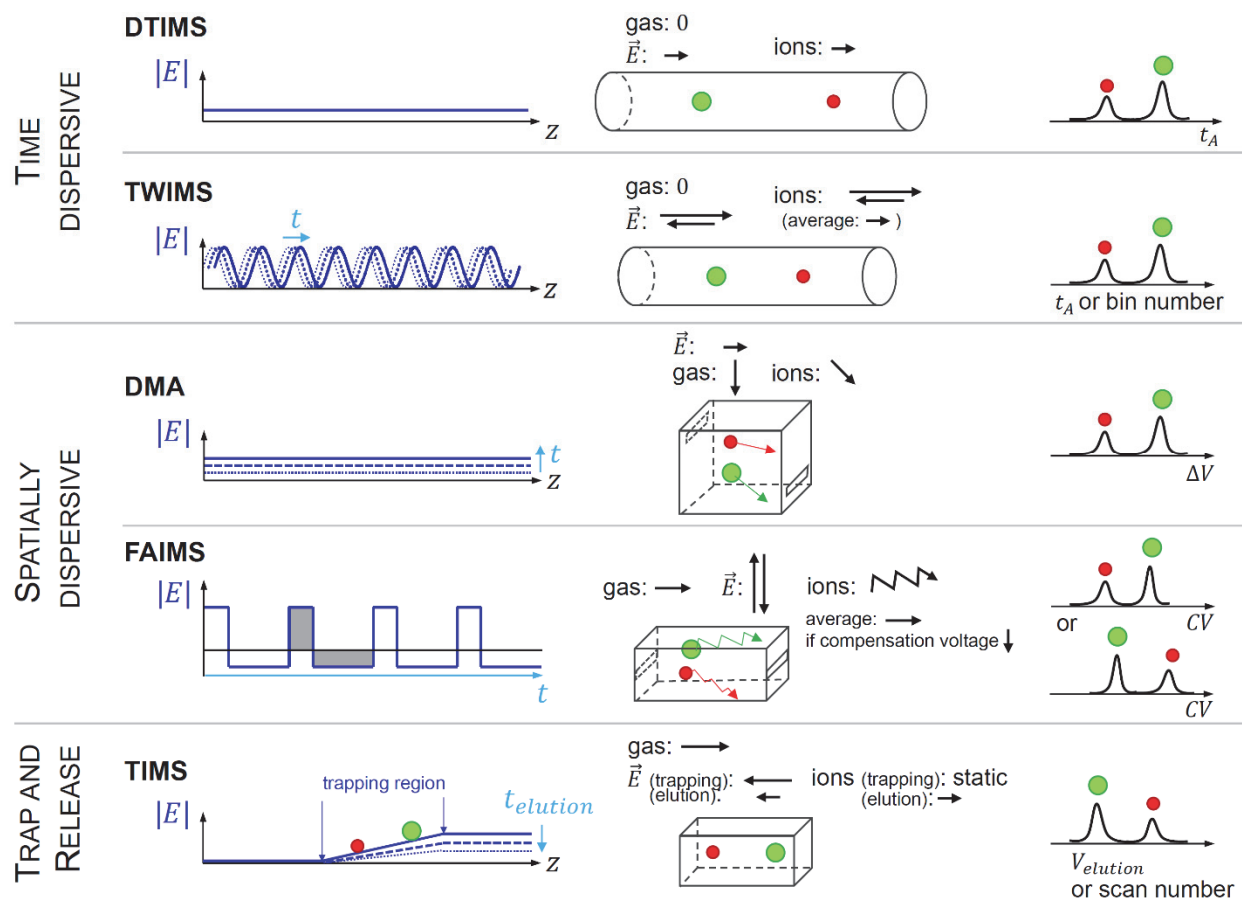


Figure 10: Classification of the most widespread ion mobility analyzers (not to scale). DTIMS = drift tube IMS; TWIMS = traveling wave IMS; DMA = differential mobility analyzer; FAIMS: field-asymmetric waveform IMS; TIMS = trapped IMS. Left: how the electric field is applied depending on instrument axis z (from entrance to exit) and on time. The electric field is constant in time only in DTIMS. Middle: schematic depiction of typical gas flows, electric fields and ion movement in each analyzer type. Note

that a gas net flow = 0 does not mean that the gas is static; the thermal agitation of the gas is not depicted. Right: typical mobility data for an ion of the same charge and different collision cross-sections.

4.1. Drift tube IMS (DTIMS)

The simplest practical realization is called a drift tube (DT) or drift tube IMS (DTIMS). A drift tube is a tube in which a uniform electric field is applied through a potential difference ΔV applied between two planes separated by a distance L . The experiment consists in measuring the time t_d required to travel this distance L .

However, even if the principle of the drift tube is simple, it is not easy in practice to determine precisely L , to measure t_d , to ensure that the electric field is perfectly homogeneous and thus that the real ΔV is known at any point of the tube, and to ensure that the gas has no net flow (due to pumping and other pressure differentials). The first-principles determination of mobilities (K or K_0) can only be realized in an ideal drift tube (see section 5.3.1). In case the drift tube realization is not obeying the above criteria, it can be calibrated with compounds of known K_0 .

4.2. Traveling wave IMS (TWIMS)

The original device consists of a stacked ring ion guide in which in addition to the axial-confining radiofrequency, traveling DC pulses are applied to push the ions from entrance to exit. The electric fields created by the DC pulses are called “waves”. If the field is high and the pressure is low (high E/N), friction is not sufficient to give rise to a mobility effect and all ions are pushed at the wave speed. However, ion mobility effects become manifest when E/N is low enough (because of higher pressure or lower field than for use as simple ion guide) that friction prevents the ions from keeping pace with the wave speed.²⁰

An advantage of TWIMS compared to DTIMS is the size of the device for comparable resolving power: the TWIMS device is shorter. One of the first applications demonstrated for TWIMS was the characterization of macromolecular protein complexes by the Robinson group.²¹ The TWIMS consequently became particularly popular for structural biology applications. However, the time the ions spend in the traveling wave ion guide is not related to the mobility through Equation (3). The above-mentioned seminal paper also introduced the notion that TWIMS could be calibrated by compounds of known collision cross-sections in order to extract information on molecular structure.

4.3. Differential mobility analyzer (DMA)

This mobility device functions as a filter: only ions of the proper mobility range are able to travel from entrance to exit and others are lost. As DMAs are operated at constant and low E/N , Equation (3) is valid, but instead of measuring a drift time, one measures the voltage difference at which ions pass through the device in order to deduce their mobility. The other difference with DTIMS is that, in DMA, the gas is not static but instead flows perpendicularly to the electric field. DMA was first developed in the context of aerosol science,²² and is still most popular in that research field.

4.4. Field-asymmetric waveform IMS (FAIMS)

The original FAIMS device had the flat-plane configuration shown in Figure 10.²³ Since then, many other instrument designs were proposed, among which was cylindrical FAIMS.²⁴ Like DMA, FAIMS devices are filters, used for separation purposes. But on the contrary to DMA, FAIMS cannot be used to determine collision cross-sections because of the high-field effects.

In practice, high field and low field pulses are applied alternatively: high field for a shorter time than low fields, with the shaded areas in Figure 10 approximately equal. Ions having different mobilities at high and low E/N will deviate from the instrumental axis, and a DC compensation voltage is required for ions to pass through the filter. The FAIMS spectra thus consist of the ion signal as a function of the compensation voltage.

4.5. Trapped ion mobility spectrometry (TIMS)

Trapped ion mobility is based on trapping the ions using a field gradient opposed to a gas flow. Depending on the frictional forces, the ions will stabilize at the position defined by an electrical force exactly compensating the frictional one.^{25, 26} Then the ions are sequentially eluted from the device by progressively lowering the gradient. TIMS devices also use axial RF confinement.

The trapping and release of ions from the TIMS device can be synchronized with the mass spectrometer, and a seminal paper by Matthias Mann's group demonstrated the benefit of Parallel Accumulation–Serial Fragmentation (PASEF) for proteomics applications, which became a main market for TIMS.²⁷

5. Comparison between ion mobility measurement techniques

5.1. Spatial and/or temporal separation

Another way to classify the mobility measurement approaches is how the ions are physically separated: in time or in space. Figure 10 shows a simplified classification of the five most widely used techniques, and more thorough discussion can be found elsewhere.²⁸

The time-dispersive techniques are the ion mobility spectrometry analogues of time-of-flight mass spectrometry. The ions are separated according to the time they spend in the mobility analyzer. The drift tube IMS (DTIMS) uses a constant and low electric field, while the traveling wave IMS (TWIMS) uses waves of electric field that travel towards the exit of the tube.

The spatially dispersive approaches give the ions different trajectories depending on their mobility. Such devices are most often used as filters, with only the fraction of the ions with the right mobility going through an exit slit at given applied voltages. They are the ion mobility spectrometry analogues of quadrupole or electric sector mass spectrometry. The differential mobility analyzer (DMA) uses a constant and low electric field, while the field-asymmetric waveform IMS (FAIMS) uses temporally alternating high and low fields.

Finally, trapped ion mobility spectrometry (TIMS) separates the ions spatially in a trapping region, then releases the ions by progressively by lowering the voltage. The elution according to ion mobility is the IMS analogue of mass-selective instability scans in ion trap mass spectrometry.

5.2. Linear vs. non-linear methods

Because of the dependency of the reduced mobility on E/N (Eq. (6)), an important distinction is between low-field and high-field methods. Low-field methods are also called “linear methods” because the measured property is linearly related to the electric field. DTIMS, TWIMS, DMA and TIMS are considered as linear methods, i.e. they are usually used and interpreted by assuming they operate in the low-field limit. In practice, the validity of this assumption depends on the fields and pressures used. The orders of magnitude of E/N for each technique are discussed in detail elsewhere.²⁹

In contrast, FAIMS relies by definition on alternating low and high fields. Consequently, (1) Equation (10) is not valid and one cannot determine collision cross-sections from such measurements, and (2) the order of ion elution is not directed by their low-field mobility. In Figure 10, this is indicated by the fact that the order of elution cannot be predicted based on ion size or charge.

5.3. Requirement for calibration

5.3.1. Drift tube IMS as a primary method of mobility measurement

A primary method of measurement is the one having “the highest metrological qualities, whose operation can be completely described and understood, for which a complete uncertainty statement can be written in terms of the SI, and whose results are, therefore, accepted without reference to a standard of the quantity being measured”.^{30, 31} Drift tube IMS instruments with no net gas flow and the ability to measure directly the drift time t_d fulfil those requirements.³² The mathematical description is given by combining Eqs. (3) and (5):

$$K_0 = \frac{L^2 \cdot p T_0}{t_d \cdot \Delta V \cdot p_0 T} \quad (11)$$

However only few instruments have the ability to measure t_d directly, and it is more common to measure an arrival time t_A , the sum of a constant time spent outside the drift region (t_0) and the voltage-dependent drift time (t_d). In such instrumental configuration, the arrival time is measured as a function of the drift voltage ΔV (or $p/\Delta V$ if the pressure can change during the experiment), and the value of K_0 is determined from the slope using Eq. (12):

$$t_A = t_0 + \left(\frac{L^2 \cdot p T_0}{K_0 \cdot p_0 T} \right) \cdot \frac{1}{\Delta V} \quad (12)$$

5.3.2. Secondary methods of measurement: calibration

DMA and TIMS also offer the possibility to determine K_0 based on first principles, but these methods do not have “the highest metrological qualities” because it is harder to account for the gas movement. Similarly, it is not easy to perform DTIMS measurements while ensuring the highest metrological qualities for measuring times, temperatures, pressures, voltage differences, and gas flow dynamics, and thus it is often more convenient to calibrate DTIMS as well. Finally, TWIMS must always be calibrated, because the description of ion movement in TWIMS is to date still incomplete.

Calibration requires available K_0 values measured using the primary method for given ion, gas, and temperature conditions, ideally mimicking those of the calibrated measurement. Figure 11 summarizes and compares the typical calibration for all four linear mobility measurement methods. Further discussion for each type of mobility analyzer will be found in the following chapters or in a recent review,⁸ but Figure 11 underlines that the calibration is theoretically linear for DTIMS and DMA, approximately linear for TIMS, and non-linear for TWIMS.

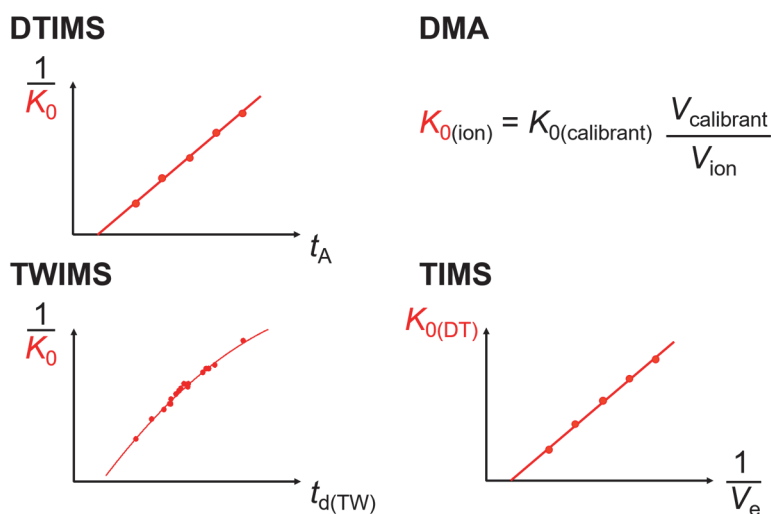


Figure 11: Typical calibration approaches for DTIMS, DMA, TWIMS and TIMS. The calibrant K_0 values come from primary measurements (typically DTIMS).

5.4. IM-MS hyphenation

When considering the merits of different ion mobility mass spectrometry instruments, it is important to consider not only the merits of the ion mobility analyzer, but of the entire instrument. Thus, attention should also be paid the qualities of the mass analyzers, the possibilities offered by the hyphenation configuration, the fragmentation methods that can be coupled to it, and the data interpretation software solutions accompanying the instrument. Here we will briefly discuss the mass analyzer hyphenation with IM in commercial implementations.

5.4.1. Mass analyzers interfaced with IM

Spatially dispersive IM devices can be operated as IM filters, and thus combined with any type of mass spectrometer, between the source and the analyzer(s).

For time-dispersive IM methods, the optimal coupling is with time-of-flight (TOF) mass analyzers, because their sampling frequency allows to collect several full mass spectra over each IM peak. Yet other implementations are possible, as shown by early implementations with quadrupoles (Q) used as mass filters,³³ or with orbitrap coupling using time-dispersive ion mobility as a filter through time gating.³⁴

Finally, the mobility-selective instability scan in TIMS can in theory be conducted at any speed and synchronized with any analyzer, but although coupling with slow analyzers (i.e., Fourier transform ion cyclotron resonance) has been reported,³⁵ the current commercial hyphenation is with a time-of-flight mass analyzer.

5.4.2. Ion mobility mass spectrometry combinations

5.4.2.1. IM-MS(/MS)

Here the mobility analyzer is placed between the source and the mass spectrometer. This is a widespread configuration. One practical reason comes from the pressure differentials: the IM pressure is indeed often in-between those of the source and of the mass analyzer. Another practical reason is that mobility separation was engineered as an “add-on” to a pre-existing mass spectrometer, and commercialized as IM-MS. Some manufacturers commercialize such add-ons, with the coupling interface for a range of ion sources from different mass spectrometry manufacturers. The absence of mass selection before ion mobility separation can be an inconvenient aspect for the ion mobility interpretation, in cases where the ions fragment or continue their declustering during or after the mobility separation, but before mass analysis: ions with the mass of the product ion can actually have been separated with the mobility characteristics of the parent ion.

5.4.2.2. MS-IM-MS

The first commercial implementation of ion mobility mass spectrometry, the Synapt™ by Waters, consisted of a Q-TOF configuration wherein the collision cell was replaced by a three-stage traveling wave ion guide, the central one being operated to perform traveling wave ion mobility separation (the other two sections serving as collision cells). In contrast with IM-MS configurations discussed in the previous section, this MS-IM-MS configuration allows one, if desired, to mass-select ions before the mobility separation. This highlights the feasibility of coupling MS and IM separations in creative manners, which then allow to implement advanced analysis workflows.

5.4.2.3. IM/IM

In 2006, Clemmer and collaborators first described “an IMS/IMS analogue of MS/MS”.³⁶ Here, ions are selected (in time) by a first drift tube, activated by collisions to cause fragmentation or conformational

rearrangements, and re-analyzed by a second drift tube. In 2009 the same group proposed the first cyclic ion mobility spectrometers, with four drift tubes arranged in a circle.³⁷ In 2019 the first commercial implementation of IMS/IMS came on the market, with the Waters cyclic IM, wherein a circular traveling wave ion guide allows the user to send time-gated pulses of ions to a collision region, and re-inject them for further IMS-MS.

6. Conclusion and outlook

Ion mobility spectrometry is currently in full swing: the mobility separation capability is now a desired feature for all high-performance mass spectrometers, while technological advances constantly increase the resolving power of mobility separation. Since its early days, IMS has often been presented as an additional method of separation. Compared to chromatography, IMS could make the difference for conformational isomers or aid charge state filtering. Now, with resolving powers over 100, soon 1000, mobility analyzers can be seen as the friction-based counterparts of mass analyzers, and both can be combined creatively.

Besides the separation capabilities that allow to decipher the composition of ever more complex mixtures, e.g. in the fields of proteomics, metabolomics, or environmental sciences, there has been much progress during the last five years towards compound identification and structural analysis based on collision cross-sections. Databases of collision cross-section values have flourished, but also the community has matured by tackling the problem of the metrology of ion mobility spectrometry.⁸ Yet further work is needed so that databases can one day be unified, and collision cross-section values will all have documented uncertainties.

Finally, it often remains challenging to interpret IM-MS data in terms of structure or conformation. This chapter introduced the fundamental concepts allowing the reader to understand the nature of the challenge. In particular, the collision cross-section is not just a unique property of a compound, akin to a melting point, but a property also depending on the gas (its nature and temperature), and on the history of the ion population from the starting analyte to the entry in the spectrometer. In the author's opinion, understanding and eventually predicting the effects of charging and internal energy (activation history) on collision cross-sections will be an important challenge for fully leveraging ion mobility measurements in structural analysis.

Acknowledgements

I would like to acknowledge my group members (Frédéric Rosu, Nina Khristenko, Eric Largy, Anirban Ghosh, Alexander König and Debasmita Ghosh) and the editors for their comments on the manuscript.

References

1. L. A. Viehland, *Gaseous Ion Mobility, Diffusion, and Reaction*, Springer Nature Switzerland AG, 2018.
2. J. Gidden and M. T. Bowers, *Eur. Phys. J. D*, 2002, **20**, 409-419.

3. L. S. Fenn, M. Kliman, A. Mahsut, S. R. Zhao and J. A. McLean, *Anal. Bioanal. Chem.*, 2009, **394**, 235-244.
4. A. Marchand, Mass Spectrometry Study of G-Quadruplex Nucleic Acids : folding Pathways and Ligand Binding Modes, PhD thesis, University of Bordeaux, 2016. <https://tel.archives-ouvertes.fr/tel-01498459>.
5. R. Pujol-Pina, S. Vilaprinyo-Pascual, R. Mazzucato, A. Arcella, M. Vilaseca, M. Orozco and N. Carulla, *Scientific reports*, 2015, **5**, 14809.
6. JCGM, 200:2012 *International vocabulary of metrology – Basic and general concepts and associated terms (VIM)*, 3rd edition edn., BIPM, 2012.
7. JCGM, 100:2008. *GUM 1995 with minor corrections. Evaluation of measurement data — Guide to the expression of uncertainty in measurement*, 1st edition edn., BIPM, 2008.
8. V. Gabelica, A. A. Shvartsburg, C. Afonso, P. Barran, J. L. P. Benesch, C. Bleiholder, M. T. Bowers, A. Bilbao, M. F. Bush, J. L. Campbell, I. D. G. Campuzano, T. Causon, B. H. Clowers, C. S. Creaser, E. De Pauw, J. Far, F. Fernandez-Lima, J. C. Fjeldsted, K. Giles, M. Groessl, C. J. Hogan, Jr., S. Hann, H. I. Kim, R. T. Kurulugama, J. C. May, J. A. McLean, K. Pagel, K. Richardson, M. E. Ridgeway, F. Rosu, F. Sobott, K. Thalassinou, S. J. Valentine and T. Wyttenbach, *Mass Spectrom Rev*, 2019, **38**, 291-320.
9. S. Warnke, J. Seo, J. Boschmans, F. Sobott, J. H. Scrivens, C. Bleiholder, M. T. Bowers, S. Gewinner, W. Schollkopf, K. Pagel and G. von Helden, *J. Am. Chem. Soc.*, 2015, **137**, 4236-4242.
10. T. Wyttenbach, C. Bleiholder and M. T. Bowers, *Anal. Chem.*, 2013, **85**, 2191-2199.
11. The expression of the Volt in SI base units is: $V = \text{kg m}^2 \text{A}^{-1} \text{s}^{-3}$. Since the Volt is related to SI units without a conversion factor, its use is accepted as a unit directly derived from the SI.
12. A. A. Shvartsburg, *Differential ion mobility spectrometry. Nonlinear ion transport and fundamentals of FAIMS*, CRC Press, Boca Raton, 2009.
13. IUPAC, *Compendium of Chemical Terminology, 2nd ed. (the "Gold Book")*. Compiled by A. D. McNaught and A. Wilkinson, Blackwell Scientific Publications, Oxford, 1997.
14. Anupriya, E. Gustafson, D. N. Mortensen and D. V. Dearden, *J. Am. Soc. Mass Spectrom.*, 2017, **29**, 251-259.
15. A. G. Elliott, C. C. Harper, H. W. Lin, A. C. Susa, Z. Xia and E. R. Williams, *Anal. Chem.*, 2017, **89**, 7701-7708.
16. J. D. Sanders, D. Grinfeld, K. Aizikov, A. Makarov, D. D. Holden and J. S. Brodbelt, *Anal. Chem.*, 2018, **90**, 5896-5902.
17. M. P. Langevin, *Ann. Chim. Phys.*, 1905, **8**, 245-288.
18. W. F. Siems, L. A. Viehland and H. H. Hill, Jr., *Anal. Chem.*, 2012, **84**, 9782-9791.
19. A. Marchand, S. Livet, F. Rosu and V. Gabelica, *Anal. Chem.*, 2017, **89**, 12674-12681.
20. K. Giles, S. D. Pringle, K. R. Worthington, D. Little, J. L. Wildgoose and R. H. Bateman, *Rapid Commun. Mass Spectrom.*, 2004, **18**, 2401-2414.
21. B. T. Ruotolo, K. Giles, I. Campuzano, A. M. Sandercock, R. H. Bateman and C. V. Robinson, *Science*, 2005, **310**, 1658-1661.
22. G. P. Reischl, *Aerosol Sci. Technol.*, 1991, **14**, 5-24.
23. R. W. Purves, R. Guevremont, S. Day, C. W. Pipich and M. S. Matyjaszczyk, *Rev. Sci. Instrum.*, 1998, **69**, 4094-4105.
24. R. Guevremont, *J. Chromatogr. A.*, 2004, **1058**, 3-19.
25. F. Fernandez-Lima, D. A. Kaplan, J. Suetering and M. A. Park, *Int. J. Ion Mobil. Spectrom.*, 2011, **14**, 93-98.
26. K. Michelmann, J. A. Silveira, M. E. Ridgeway and M. A. Park, *J. Am. Soc. Mass Spectrom.*, 2015, **26**, 14-24.

27. F. Meier, S. Beck, N. Grassl, M. Lubeck, M. A. Park, O. Raether and M. Mann, *J. Proteome Res.*, 2015, **14**, 5378-5387.
28. J. C. May and J. A. McLean, *Anal. Chem.*, 2015, **87**, 1422-1436.
29. V. Gabelica and E. Marklund, *Curr. Opin. Chem. Biol.*, 2018, **42**, 51-59.
30. T. J. Quinn, *Metrologia*, 1997, **34**, 61-65.
31. BIPM, *Com. Cons. Quantité de Matière*, 1995, **1**, Q5.
32. B. C. Hauck, W. F. Siems, C. S. Harden, V. M. McHugh and H. H. Hill, *Int. J. Ion Mobil. Spectrom.*, 2017, **20**, 57-66.
33. T. Wyttenbach, P. R. Kemper and M. T. Bowers, *Int. J. Mass Spectrom.*, 2001, **212**, 13-23.
34. Y. M. Ibrahim, S. V. Garimella, S. A. Prost, R. Wojcik, R. V. Norheim, E. S. Baker, I. Rusyn and R. D. Smith, *Anal Chem*, 2016, **88**, 12152-12160.
35. L. V. Tose, P. Benigni, D. Leyva, A. Sundberg, C. E. Ramirez, M. E. Ridgeway, M. A. Park, W. Romao, R. Jaffe and F. Fernandez-Lima, *Rapid Commun. Mass Spectrom.*, 2018, **32**, 1287-1295.
36. S. L. Koeniger, S. I. Merenbloom, S. J. Valentine, M. F. Jarrold, H. R. Udseth, R. D. Smith and D. E. Clemmer, *Anal. Chem.*, 2006, **78**, 4161-4174.
37. S. I. Merenbloom, R. S. Glaskin, Z. B. Henson and D. E. Clemmer, *Anal. Chem.*, 2009, **81**, 1482-1487.