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Fundamentals of ion mobility spectrometry

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Fundamental questions in ion mobility spectrometry have practical implications for analytical applications in general, and omics in particular, in three respects. (1) Understanding how ion mobility and collision cross section values depend on the collision gas, on the electric field and on temperature is crucial to ascertain their transferability across instrumental platforms. (2) Predicting collision cross section values for new analytes is necessary to exploit the full potential of ion mobility in discovery workflows. (3) Finally, understanding the fate of ion structures in the gas phase is essential to infer meaningful information on solution structures based on gas-phase ion mobility measurements. We review here the most recent advances in ion mobility fundamentals, relevant to these three aspects.

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Introduction

Omics sciences require to separate, identify and quantify all compounds in a mixture. Ion mobility spectrometry (IMS), in which electric fields are used to drag analytes through a buffer gas, is useful for separation sciences, and can also aid identification. We review here the fundamental principles behind using IMS for identification and structural characterization. Although theoretical foundations were laid decades ago [1,2], fundamental contributions have flourished in the last two years. We gather contributions of prime importance for IMS, spanning from small molecules (lipidomics, metabolomics) to large multi-protein assemblies (structural proteomics and native mass spectrometry).

In IMS, the force exerted by an electric field on an analyte ion is exactly balanced by friction with the buffer gas, yielding a steady-state analyte velocity v_d. The ion mobility K (Eq. (1)) is thus a measure of friction linked to an observable: the time t_d the ions take to traverse the length l of the mobility cell.

$$K = \frac{v_d}{E} = \frac{t_d}{lE} \tag{1}$$

K depends on the collision frequency, hence on the gas number density (N), gas temperature (T) and pressure (p), so the reduced mobility $K_0 = K \cdot N/N_0 = K \cdot (p/p_0)/(T_0/T)$ is better to compare different experiments (in standard conditions, $N_0 = 2.687 \times 10^{25}$ m^{-3}, $p_0 = 760$ Torr, $T_0 = 273.16$ K). When v_d is small compared to the ion thermal velocity v_T, K can be expressed as Eq. (2) [1].

$$K = \frac{3}{16}\sqrt{\frac{2\pi}{\mu k_B T}} \times \frac{ze}{N\Omega} \tag{2}$$

μ is the reduced mass of the ion–gas pair ($\mu = mM/(m + M)$, where m and M are the ion and gas-particle masses), k_B is the Boltzmann constant, and ze is the analyte charge.

Ω, often called the 'collision cross section' (CCS), is actually a 'momentum transfer collision integral', that is, the momentum transfer between ion and gas particles averaged over all gas-ion relative thermal velocities. While the terms tend to be used interchangeably in IMS, they are in fact not identical in a wider context. Scattering or dephasing measurements carried out at very low pressures, wherein collisions eject the ions from stable trajectories [3–6], allow to determine true scattering collision cross sections, which can be adequately calculated by a projection approximation. Ion mobility is different: we still detect the ions after they had undergone collisions. The momentum transfer collision integrals measured in ion mobility are different, and require taking into account the effects of the gas on the ion momentum (i.e. velocity). Although CCSs and momentum transfer collision integrals are related, they are thus not necessarily identical, and further work is warranted to bridge the gap between the two types of experiments.

In ion mobility (Eq. (2)), Ω has the dimensions of a surface, is a property of the ion–gas pair, and also depends on other parameters influencing the ion–gas collision velocities, i.e. on the temperature T, on the electric field E and on the pressure p (which controls N), and

specifically on *E/N*. The first section will review the effects of gas, field and temperature, which are crucial to interpret the data correctly, and to understand differences between instrumental setups.

IMS practitioners have three main ways to characterize the analytes: t_d (in practice, the arrival time at a detector, t_A), K_0, and Ω. Each of these values can aid identification of '*known knowns*' (molecules anticipated by the researcher, by comparison with a measured database) or of '*known unknowns*' (compounds that are unknown to the researcher, but described in the literature, by comparison with a predicted database). As t_d or t_A values depend on the instrument and on experimental conditions, they have only in-house utility. In contrast, databases of mobilities or cross section values are in principle transferrable, and the conditions for their transferability across instrumental platforms will be discussed below. Moreover, Ω can aid the identification of '*unknown unknowns*', by comparing values predicted from putative candidate structures. The fundamentals of CCS calculation will be covered in the second section. Finally, we highlight recent examples of how IMS measurements and modeling shed a new light on one of the most fundamental questions of mass spectrometry: how the structure in the gas phase relates to those in solution.

Effect of drift gas on collision cross sections

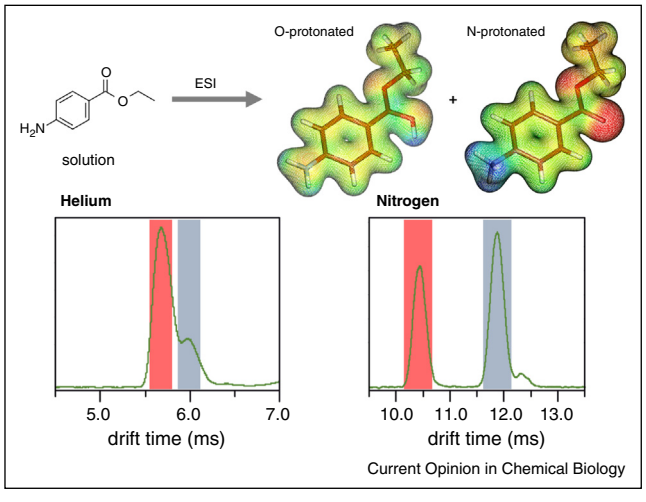
Early IMS for structural elucidation was carried out in drift tubes (DT), operated in helium because calculations are easier. Using IMS for omics became possible with the introduction of commercial high-performance electrospray IMS mass spectrometers, usually operated in nitrogen. The first commercial IMS (the Synapt HDMS^{TM}, introduced by Waters in 2006) operates with traveling wave (TW) IMS [7]. Because the electric field is not static in TWIMS, apparent drift times have not the same meaning as drift tube t_d values. An empirical correlation was made to match arrival times based on helium drift-tube CCSs [8], and recently the t_d of peptides and proteins could be modeled at low wave velocities directly from K without calibration [9].

Experience, however, showed that TWIMS calibration is not universal: CCS values depend on the nature of the calibrant, which should have size, charge, and chemical class similar to the analytes (doing so results in an average deviation of <2% between ${}^{TW}\Omega_{N2\rightarrow He}$ and ${}^{DT}\Omega_{He}$ [10]). Similarly, native protein complexes should be used for structural proteomics [11]. However, Konermann's group recommends using denatured proteins even for native protein analytes, because denatured proteins do not change CCS values when changing pre-IMS activation conditions [12]. Recently, the Robinson group also showed that native soluble proteins are inappropriate calibrants for native membrane proteins [13]. A hot question for TWIMS users is thus: '*What makes a calibrant suitable for my analytes?*'

Helium drift tube CCS values are often used to calibrate TWIMS instruments operated in nitrogen, so let's first discuss the effects of the drift gas on the CCS. Benzocaine will serve as textbook example for small molecules. In positive-mode electrospray, benzocaine forms two tautomers (different proton location, but same conformation) [14••]. They are readily separated by IMS in nitrogen, but nearly overlap in helium (Figure 1). At 300 K, interactions between the ions and helium are akin to collisions with a hard sphere, hence the mobility difference is small. In contrast, nitrogen is polarizable, and interacts more strongly with the more polar tautomer [15]. The proportionality factor between helium and nitrogen CCS values thus depends on the chemical nature (here, charge location) of the analyte, and this effect is strongest for small ions.

Recent simulations of how Ω depends on the gas temperature and identity highlight how the trends depend on the ion charge [16] and shape [17••] (Figure 2). Ω increases when the temperature decreases, because long-range interactions become more dominant at reduced v_T [18,19]. At high temperature, the impulses from 'grazing' gas particle collisions are smaller in magnitude as high thermal velocity allows little time for the interatomic forces to act, and Ω approaches the hard-sphere limit. The Ω-difference between gases at high temperature is due to the gas-particle radius, and correlates with ion size and shape [17••]. However at lower temperatures, *including room temperature*, the difference between He and N_2 is further influenced by long-range

Figure 1



The O-protonated and N-protonated forms of benzocaine, produced simultaneously by electrospray in acetonitrile, separate differently in helium or nitrogen drift tube ion mobility (image courtesy of Kevin Pagel [14••]).