Resolution Equations for High-Field Ion Mobility

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An extension of current mobility resolution equations as they apply to high-field ion mobility spectrometry is presented. The new resolution expression is applied to arrival time distributions for ions having a large range of ion mobilities and mass-to-charge ratios (m/z). The results indicate that the new equation can be utilized to predict the mobility resolution over a broader range of applied electric fields than previous ion mobility resolution expressions. (J Am Soc Mass Spectrom 2004, 15, 1320–1324) © 2004 American Society for Mass Spectrometry

Separation based on the mobility of an ion through a neutral buffer gas (i.e., ion mobility spectrometry (IMS)) [1, 2] is an important technology for studying long-lived electronic states of gas-phase ions and a sensitive method for detection of air-borne species [3, 4]. Recent work, which demonstrates the utility of IMS for analyzing gas-phase ionized biopolymers (i.e., peptides, proteins, DNA, etc.), has renewed interest in the analytical applications of IMS and led to the development of several high-resolution drift tubes in combination with mass spectrometry [5–11]. In order to perform a gas-phase separation of near thermal, structurally isomeric gas-phase ion populations, most IMS separations are performed near the low-field limit. Low-field conditions are important for studies of gas-phase peptide/protein structure because collisional activation can, in some cases, lead to structural rearrangement and/or fragmentation.

In addition to the distinctions drawn for IMS operation in terms of separation field strength, the use of IMS can be divided into two pressure regimes, viz. high pressure (typically low-field) and low pressure (either low or high applied fields), and there are distinct advantages associated with both pressure regimes. At high pressure, the number of ion-neutral collisions is high, i.e., C_{60}^{+} undergoes 10^{10} collisions/s at 760 torr He compared with about 10^7 collisions/s for the same ion at 1 torr He. It is also important to consider that collisions with low-level impurities may significantly influence the separation and total drift time of an analyte ion. For example, in a 30 cm drift cell maintained at 760 torr He, C_{60}^{+} will undergo 10^5 – 10^6 collisions with a 0.01% impurity, compared with ca. 10

collisions with the same level of impurity at 1 torr He. Another advantage of low-pressure drift tubes is the ease of coupling ion mobility with high vacuum mass spectrometers. Lastly, low-pressure (or high-field) mobility decreases ion separation times, leading to higher throughput analysis (approximately 3 orders of magnitude faster for analysis times at 1 torr over 760 torr).

Low-field mobility conditions are defined in terms of the kinetic energy acquired by the ion in the presence of an applied field (E_0). That is, the translational energy gained by the ion between collisions should be less than the thermal energy of the collision gas [12, 13],

$$\left(\frac{m}{M} + \frac{M}{m}\right)eE_0\lambda \ll k_bT \quad (1)$$

where m is the mass of the ion, M is the mass of the neutral drift gas, e is the charge on the ion, k_b is Boltzmann's constant, and T is the collision gas temperature. The ion-mass dependence (and ion-size dependence) of the above inequality is described by expressing eq 1 in terms of drift gas pressure (p) and ion diameter (d) rather than the mean free path (λ).

$$\frac{E_0}{p} = 0.345 \frac{E_0}{N} < \left(\frac{m}{m+M}\right)^{1/2} \frac{d^2}{z} \quad (2)$$

where N is the number density of neutral gas molecules, and z is the number of equivalent charges on the ion [2, 12, 13]. The factor for conversion between E/p (in $V \text{ cm}^{-1} \text{ torr}^{-1}$) and E/N (in Td) is given in eq 2 assuming room temperature. Eq 2 can be used to establish the low-field limit for a range of masses, pressures, and applied electric fields. Although the low-field limit is generally assumed to be $\sim 2 \text{ V cm}^{-1} \text{ torr}^{-1}$ for the separation of atomic species, the

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same threshold for peptide ions of m/z 500–2500 ranges from 20–45 V cm⁻¹ torr⁻¹ [14].

This report describes ion mobility resolution under high-field conditions by applying Wannier's diffusion relation to the known expression for ion mobility (IM) resolution. The resulting equation is applicable to IM separations over a much broader range of field conditions. A detailed examination of this new expression suggests that resolution for ions that have low mobilities (i.e., large protein and peptide ions) approach the theoretical low-field resolution, even at high E/p , where small ions exhibit a decrease in resolution. Also, Wannier's diffusion relations indicate that radial diffusion under high-field conditions will be greater than low-field conditions, leading to a decrease of ion transmission in the high-field limit. We examine both figures of merit, resolution and transmission efficiency, in the evaluation of IM separation performed at high E/p .

Theory

The mobility (K) of an ion through a neutral drift gas is determined by the ion's drift velocity (v_d) and applied electric field [13];

$$v_d = \frac{L}{t_d} = KE_o \quad (3)$$

where the ion's mobility (K) is described by Revercomb and Mason [15] to be inversely proportional to the reduced mass (μ) of the ion and neutral drift gas, and the average collision cross-section (Ω).

$$K \propto \frac{1}{\mu^{1/2}\Omega} \quad (4)$$

Ion Mobility also varies as a function of temperature and pressure, thus the reduced mobility (K_o , corrected to standard temperature and pressure) is typically reported. Mobility resolution has been derived for low-field conditions [16–19]; however, high applied fields have a distinct effect on mobility resolution.

The mobility peak width depends on four broadening terms: the initial pulse, diffusion, space-charge effects, and ion-molecule reactions [17, 18]. If we assume that the initial ion gate width is narrow with respect to the peak width, the number of ions in each ion packet is low, and ion-neutral interaction potentials with the drift gas are negligible, then we can assume that band broadening is limited to the diffusion term. A general diffusion profile can be obtained by solving Fick's second law of diffusion [7] provided that the diffusion coefficient (D) is constant for a particular system (no pressure gradients) [20]. If we assume that ion diffusion is equal in both directions along the z axis [20], Fick's second law can be utilized to solve for eq 5:

$$R = \frac{L}{3.32 \sqrt{Dt_d}} \quad (5)$$

where the resolution (R) depends on the drift length, diffusion constant of the ion, and drift time experienced by the ion (t_d) [16–19]. By solving eq 3 for the drift length and substituting the new expression into eq 5, we can evaluate the effect of mobility, diffusion, and applied electric field on IM resolution [16].

$$R = \frac{KE_o}{3.32} \sqrt{\frac{t_d}{D}} \quad (6)$$

Under low-field conditions, the diffusion coefficient is accurately described by the Nernst-Einstein-Townsend relation [16].

$$D = \frac{k_b T}{e} K \quad (7)$$

where e is the charge, k_b the Boltzmann constant, and T is the temperature. If we assume the temperature for both the bath gas and the ion are the same, i.e., ions are within the low-field limit, then the resolution equation simplifies to [16]:

$$R = 32.33 \sqrt{\frac{LE_o z}{T}} \quad (8)$$

At high field strengths, Wannier used the Boltzmann equation and an isotropic scattering model to describe ion diffusion through a neutral drift gas [12, 13, 21].

$$D_t = \frac{k_b T}{e} K + \frac{M}{3} \cdot \frac{M + 3.72m}{M + 1908m} \cdot \frac{E^2 K^3}{e} \quad (9)$$

In order to evaluate the influence of high field strength on resolution, eq 6 was combined with Wannier's relation (eq 9). To reduce the number of terms in Wannier's relation, we condensed the mass term to μ_w (eq 10) to obtain eq 11.

$$\mu_2 = \frac{M}{3} \frac{M + 3.72m}{M + 1.908m} \quad (10)$$

$$D_t = \frac{k_b T}{e} K + \mu_w \cdot \frac{E^2 K^3}{e} \quad (11)$$

Equation 11 is substituted into eq 6 and simplified to produce eq 12.

$$R = 0.30 \left(\frac{eLE}{k_b T + \mu_w E^2 K^2} \right)^{\frac{1}{2}} \quad (12)$$

Eq 12 can be used to accurately predict the diffusion-

limited peak broadening experienced by an ion at both high and low field strengths.

Experimental

Ion mobility data was acquired using a matrix-assisted laser desorption ionization (MALDI)-ion mobility (IM)-orthogonal- time-of-flight (o-TOF) mass spectrometer built in-house and described elsewhere [22]. Briefly, ions are created at the operating pressure of the drift tube (1 torr He) and allowed to drift over 45 cm in the presence of a superimposed linear and non-linear electric fields [18]. Ions that exit the drift cell are mass analyzed using a 30 cm orthogonal TOF. Data is collected using a custom data acquisition software package (Ionwerks, Inc. Houston, TX) and processed using Transform (IDL, Research Systems, Boulder, CO). C_{60}^+ and bradykinin were purchased from Sigma-Aldrich (St. Louis, MO) and used without additional purification. MALDI was performed by diluting a 100 picomole/ μL stock solution of bradykinin with a solution of α -cyano-4-hydroxycinnamic acid to a matrix-to-analyte ratio of 2000:1.

Temperature-controlled studies performed on low mass ions ($m/z < 100$) were carried out on a liquid nitrogen-cooled ion mobility orthogonal- time-of-flight mass spectrometer built in-house [23]. The samples are ionized using electron impact and separated in the drift tube at a constant drift gas pressure of ~ 1 torr at room temperature. The data acquisition package is the same as for the MALDI-IM-o-TOF instrument described above.

Unknown mobility, resolution, diffusion, and transmission values were calculated using a predictive software package called "MobCross" developed at Texas A&M University. This package utilizes known and derived equations to calculate mobility values. It also employs a Monte Carlo simulation to predict ion cross-sections based on ab initio and molecular mechanics calculations [24].

Results and Discussion

Wannier's relation predicts that the diffusion experienced by an ion changes as a function of the square of the applied field, but also as the cube of the mobility of the ion. The deviation between the two diffusion relations for C_{60}^+ (720 m/z , $K_o = 4.31$) begins at about 2 $\text{V cm}^{-1} \text{ torr}^{-1}$ and exceeds three orders of magnitude at fields greater than 40 $\text{V cm}^{-1} \text{ torr}^{-1}$. Figure 1a shows the predicted effect of field strength (E/p) on resolution based on eq 8 (upper trend, dashed line) and eq 12 (lower trend, solid line). Because the Nernst-Einstein relation is independent of applied field, the resolution calculated using eq 8 can be significantly different than the resolution calculated using eq 12 for the same ion. For example, at 60 $\text{V cm}^{-1} \text{ torr}^{-1}$ the calculated resolution for C_{60}^+ would be 45% of the predicted Einstein relation value. Note that in Figure 1a the resolution

Figure 1. (a) Plot of predicted resolution versus applied field for C_{60} at 1 torr drift pressure. (b) The resolution versus reduced mobility of a 15cm drift cell at 1 torr for applied fields of 10, 20, and 50V/cm.

values for C_{60}^+ reach a maximum value and then reduce as the applied field is increased; however, this local maximum changes as a function of ion mobility. Figure 1b illustrates that, for ions having a large mobility (K_o), an increase in E/p results in a decrease in resolution, whereas for large ions (i.e., ions having a low mobility), the resolution increases as E/p is increased. Ions having an intermediate mobility value (i.e., C_{60}^+) display a cross-over point that shows an increase in resolution up to a point, beyond which the resolution decreases. Furthermore, the predictions shown in Figure 1a and b indicate that mobility resolution for large ions can be estimated using eq 8 even at high E/p .

Eq 12 accurately predicts resolution for varying field conditions and for a broad range of ions. For example, it is well known that IM resolution is observed to decrease for small ions ($m/z < 100$) as the applied field is increased ($\sim 40\%$ for He^+ in He drift gas for a transition from 2 $\text{V cm}^{-1} \text{ torr}^{-1}$ to 10 $\text{V cm}^{-1} \text{ torr}^{-1}$). Eq 8 does not predict this decrease in resolution as a function of increased applied field, whereas the field dependant resolution is predicted by eq 12 (see Figure 1). It is important to note that the contribution of the ion-neutral interaction is not negligible in this case, due to a charge-exchange mechanism operative at higher field strength conditions [23].

Figure 2. Plot of experimental C_{60} resolution at varying E/p compared to the resolution predictions from eq 6 (low field) and eq 12.

Figure 2 reproduces the low field and high field approximations shown in Figure 1a, and experimental data for C_{60}^+ has been added for comparison. Within the error of the resolution measurement (expressed as a range over multiple runs at each field strength), the measured resolution more closely matches the predicted value from eq 12 (bottom) than those obtained using eq 8 (top). Resolution for large molecules (low mobility) can be estimated using eq 8, suggesting that resolution increases at higher applied fields for ions with $K_0 < 1$ (as is observed experimentally). Utilizing the well-established mass-mobility correlation for a series of homologous ions [2], the data presented in Figure 3, a plot of drift time versus m/z for a mixture of cesium iodide, C_{60} , and C_{70} , supports the theoretical results presented in Figure 1b. That is, ions that have a lower

Figure 3. Drift time- m/z plot of a mixture Cs^+ (Signal I), C_{60}^+ (Signal II), C_{70}^+ (Signal III), and other related carbon clusters (signals for C_{110} – C_{132} ions are labeled as, and signals for C_{164} – C_{186} ions are labeled as V) acquired at $66 \text{ V cm}^{-1} \text{ torr}^{-1}$.

mobility will have a higher IM resolution than ions with higher ion mobility at the same applied field. The measured IM resolution for three different peaks (I: Cs^+ , II: C_{60} , III: C_{70}) and the average mobility resolution for two groups of signals (IV: C_{110} – C_{132} and V: C_{164} – C_{186}) at increasing m/z are compared to theoretical resolution values from eq 8 and 12. IM resolution ($t/\Delta t$) values for signals I, II, and III are 17.5, 30.3, and 33.0 respectively. Average IM resolution values for the ion signals in regions IV and V of the plot are 35.8 and 41.1 respectively, thus verifying the trend in IM resolution as a function of ion mass (and mobility) predicted by eq 12.

Conclusions

Eq 12 presents an effective framework for estimating the mobility resolution at any field strength, and suggests that there are no adverse effects (in terms of resolution) for the separation of high mass, low mobility, species performed at elevated E/p values. In addition, recent results from our laboratory also suggest that the K_0 values for peptide ions in the range of 500–2500 are relatively unaffected by increased field strength up to $66 \text{ V cm}^{-1} \text{ torr}^{-1}$ ($\sim 200 \text{ Td}$) [25]. In most cases where the field-strength dependant mobility of large ions has been investigated, a slight mass dependant decrease in the apparent ion mobility is typically observed at high field strengths [26]. According to the theoretical constructs presented here, a decrease in ion mobility will only aid the acquisition of low-field, diffusion-limited resolution. Monte Carlo simulations performed in our laboratory indicate that the transmission efficiency decrease due to increased radial diffusion under high-field separation conditions is minimal for peptides and proteins, and that ion losses due to diffusion can be further mitigated by the application of ion guide-type IM drift tube designs [22, 27, 28]. In general, the results presented here clearly illustrate that operating IM separations at high E/p facilitate faster separation times, i.e., higher throughput, without loss in resolution for applications in proteomics.

Clearly, resolution is an important figure of merit for separation optimization; however a more important figure of merit is resolving power (or the ability to resolve two closely related ion signals). As IM resolution and IM resolving power are directly related, it follows that IM resolving power is unaffected at elevated E/p as well, assuming that the elevated field strength employed does not cause gas-phase structural isomerization. In addition, although eq 12 predicts that larger molecules, i.e., proteins, will approach the diffusion-limited resolution at high field strengths, the resolution for such molecules may be limited due to conformational effects. According to eq 12, decreased temperature can also be employed to increase both resolution and resolving power at high field strengths, and several research groups have observed this effect experimentally [13, 23].

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