Experimental Validation of an Ion Simulation Model for Traveling Wave Ion Mobility Spectrometry

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ABSTRACT. Ion mobility is an analytical separation technique that separates molecules (analytes) through differences in collisions between ionized gas-phase analytes and neutral gas molecules. Each analyte ion having a different intrinsic mobility in a characteristic drift-gas allows for temporal separations. Ion mobility spectrometry (IMS), frequently paired with mass spectrometry, allows separation on a molecular basis and serves as a detection tool in all ranges of scientific research. IMS can be simulated using a software program that calculates the trajectory of ions in an electric field and under gas collisions. This program, SIMION, can simulate a specific type of ion mobility known as TWIMS (Traveling Wave Ion Mobility Spectrometry) [1]. TWIMS differs from conventional DTIMS (Drift Tube Ion Mobility Spectrometry) due to TWIMS utilizing shifting potential waveforms to drive ions against a drift-gas rather than with a static electric potential gradient. This study seeks to determine the accuracy of these TWIMS computer simulations in light of experimental data to automate settings selection, a time-consuming and subjective task. Resolving power values and drift time distributions were compared between simulation and experiment to determine the accuracy of the model. Qualitative similarities in plot features were observed when comparing theoretical and empirical resolving power curves. Results show that SIMION electrodynamic simulations are accurate models of real life behavior and can be used to optimize separations.

INTRODUCTION.

Modern science is dependent on the acquisition and analysis of complex sets of quantitative data. Such as the case with separating complex compounds like DNA base pairs using gel electrophoresis, analytical technologies have advanced to allow researchers to directly observe the atomic nature of these compounds [3]. Ion mobility is one such analytical technique used to separate ions in the gas phase and is frequently paired with mass spectrometry for combining ion separations with mass measurements [3]. There are two primary types of ion mobility, which are illustrated in Figure 1. Conventional Drift Tube Ion Mobility Spectrometry (DTIMS) utilizes a static linear potential gradient (a uniform electric field) to drive ions axially through a drift tube filled with a neutral drift gas, such as helium or nitrogen (Figure 1A) [5]. The second ion mobility type is a newer technology known as Traveling Wave Ion Mobility Spectrometry (TWIMS) which has been developed into a commercial instrument [2]. TWIMS uses pulses of low potential (traveling waves) in a neutral drift gas to drive ions forward so larger ions are caught while lighter ions are carried, separating ions based on size related mobilities (Figure 1B). In TWIMS, the neutral drift gas is used to retard the forward motion of the ion caused by the traveling wave, which results in an ion mobility temporal separation that is qualitatively similar to conventional DTIMS spectra.

While TWIMS is more complicated than DTIMS, requiring many settings to be fine-tuned in order to elicit the highest separation levels, it offers many advantages. For example, TWIMS is capable of higher resolution separations than comparable length DTIMS drift cells, and is easier to adapt to mass spectrometers, since TWIMS cycles from 0-40 volts as opposed to DTIMS which typically operates at static voltages around 1000 V [2]. TWIMS has gained adoption by the analytical community, and so the validation of a theoretical simulation model capable of predicting TWIMS performance will have a broad impact to

researchers as a previously time-consuming task can be essentially automated, thereby removing a hurdle for widespread TWIMS adoption.
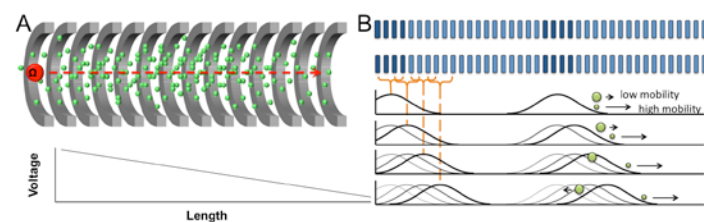

Figure 1: Panel A shows the method of operation for DTIMS in which an ion of apparent collision cross section encounters neutral drift gas molecules while being driven forward axially with a gradient of voltage. Panel B shows the method of operation in TWIMS, the top section represent the electrodes that are selectively turned on to emulate a wave-like effect of traveling potentials as represented by the bottom portion. Low mobility ions are impeded and roll over the wave whereas higher mobility ions are moved along with the wave allowing for temporal separation. Figure adapted with permission from reference 1 [1]nitrogen, and argon.

A previous study used the SIMION package of software with a custom written user program to simulate the shifting wave potentials of TWIMS [1]nitrogen, and argon. This previous study simulated TWIMS under different operational conditions in order to predict performance of the TWIMS instrument across a wide range of settings. The goal of this study was to determine the accuracy of this user created program for potential use in determining the optimal operation conditions for a TWIMS instrument, thereby increasing the efficiency of the separation. While TWIMS settings can be manually shifted, the automation of this process will save resources and make TWIMS a more practical analytical technique to researchers in all areas of science. This study used a commercial TWIMS instrument (Waters Synapt G2) to perform experiments similar to those in the simulations to evaluate the accuracy of the model. Three ion systems were tested and simulation (theory) results were compared with experimental data to evaluate the correlations in terms of the instrument sensitivity and resolving power.

MATERIALS AND METHODS.

Computer Simulations

The ion simulation program, SIMION 8.1 (Scientific Instrument Services, Ringoes, NJ) utilizing a commercial TWIMS instrument geometry (Synapt G2, Waters Co., Manchester, UK), was used to simulate ion trajectories. To simulate the TWIMS potential waves, a custom written electrodynamic program (lua programming language) developed from a previous study was modified to generate data [1]. Three different ion systems were modeled (1) benzoylmethylecgonine (cocaine), (2) 3, 4-methylenedioxy-N-methylamphetamine, (MDMA), and (3) alpha-methylphenethylamine (amphetamine), in order to determine the effects of different intensive molecular properties on resolving power. These ion systems were utilized due to their stability and precise metrics like Collision Cross Section due to their need to be detected for defense and safety purposes. Additionally, the simulated TWIMS settings were varied to determine the effect of different parameters on resolving power and sensitivity; settings evaluated included the drift cell pressure, ion system, traveling wave velocity, and initial ion gate width.

Experimental Configurations

A second generation TWIMS instrument (Synapt G2) was used to acquire data for comparison against simulated data. This TWIMS instrument utilized nitrogen as the ion mobility buffer gas and all samples were infused into an electrospray ionization source through an auxiliary sample inlet (lockspray calibration port) for a standard acquisition time of 3 minutes except at lower wave velocities (250, 300, and 350 m/s) in which low sensitivity was exhibited and acquisition time was extended to 5 minutes. Acquisitions were taken over a period of 3-5 minutes and made into a sum of several 1 Hz scans to suppress noise and amplify true signal. Data was acquired using vendor-supplied software (MassLynx, Waters).

Data Analysis

All raw data files were imported to the program Driftscope (Waters Corporation, Milford, MA) and visualized as a 2-dimensional mass-to-charge (m/z) vs drift time spectra. Corresponding analyte mass ranges were selected based on the drift spectra. Using Driftscope, this spectrum was snipped to retain the selected mass range of interest with the resulting drift time function appended to it and exported into Masslynx where a chromatogram list was generated which allowed statistical values (peak centroid and peak width) to be extracted. Resolving power was calculated from the average drift time per ion acquisition (centroid of the distribution), and the peak width was calculated as the full width at half maximum (FWHM). Due to the disparate nature of the data sets, the true average and standard deviation were supplanted with the weighted average and standard deviation. Under the assumption that the data fits a Gaussian curve, the following formula can be used to calculate the FWHM (Δt):

$$\Delta t = 2\sigma(2\ln2)^{1/2} \tag{1}$$

The FWHM computed from equation 1 can be used to calculate the resolving power (RP) of the separation using the following formula:

$$RP = t/\Delta t \tag{2}$$

Resolving power is the ability of an instrument to distinguish small or closely adjacent images and thus a helpful metric in determining the efficiency of the separation. Here, t is the average drift time and Δt is the FWHM. The area underneath the curve was estimated as a triangle using the standard area formula of a triangle, the height was taken at the highest ion count on the plot with the bases being qualitatively estimated and used as the scaling factor. The Gaussian curves were scaled by this factor to correct for the normalization in the theoretical plots. The corrected Gaussian fit was then plotted with the according raw data points for visual comparison between empirical and theoretical data sets.

RESULTS.

Resolving Power

To determine model accuracy, all data were plotted in terms of resolving power, which compares the ability of the peaks to become resolved in the presence of closely spaced signals. Because RP uses the width and the centroid of the peak (equation 2), this measurement serves as a useful metric for not only determining the efficiency of the separation but also determining abnormalities in individual component (numerator and denominator) values. Figure 2 shows the resolving power plots that were created using the drift time centroid and FWHM obtained from both the ion simulations (Figure 2A, B, and C) and the experimental measurements (Figure 2D, E, and F).

Sensitivity

While resolving power is the major concern in this study, it is also important to study the effects that different settings have on sensitivity. Figure 3 shows a comparison between experimental and simulated ion mobility spectra for the three compounds investigated. To represent the distribution of the sensitivity of ions detected, the chromatogram list (ion counts generated by software) was plotted and fitted with a Gaussian curve so that spectral width and height can be quantitatively extracted from the experimental data. Since the data sampling

resolution depends on the wave velocity, some ion mobility chromatograms showed a poor distribution shape, for example, the 400 m/s data is less defined (7 data points) than the 1000 m/s data (*ca.* 14 data points) in Figure 3. To address this concern, a Gaussian curve is used to smooth the experimental distributions and interpolate points on the graph, thus increasing the sampling resolution.
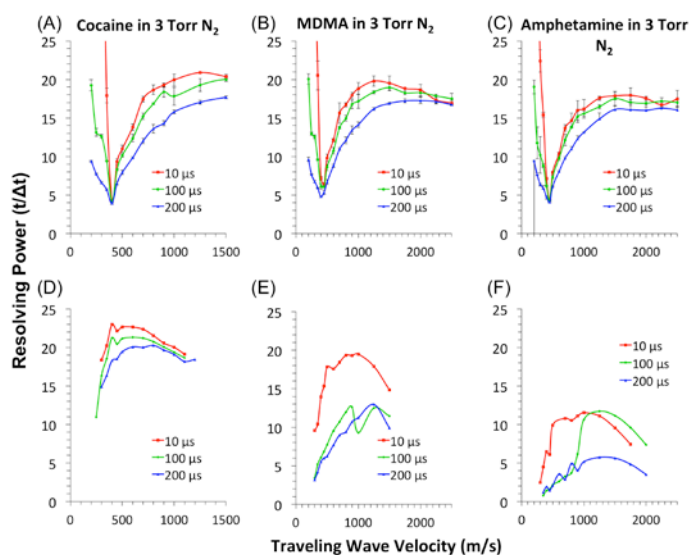

Figure 2: These resolving power plots (resolving power vs. traveling wave velocity) compare RP values between the theoretical (panels A, B,C) and experimental (Panels D,E,F) observations. All six sets of data represented here have maxima and minima and general plot features (minima and maxima) between both sets of data do in fact line up, thus showing a qualitative correlating between the theory and experiment. Plots B-E and C-F match well in terms of the location of maximum and minimum RP, showing the accuracy of the model. Plots A-B also show similar trends but may be slightly off due to Cocaine being the heaviest molecule tested, thus inefficiencies may have been magnified.
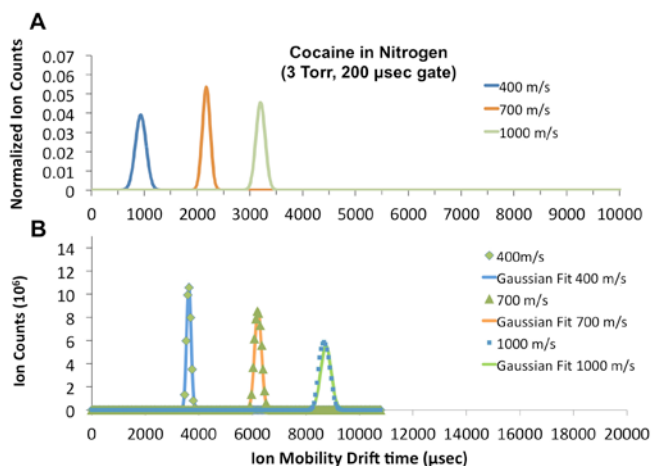

Figure 3: Panel A shows a Gaussian distribution of ion intensity for *in silico* data obtained from the ion simulations (Adapted with permission from reference [1] nitrogen, and argon). Panel B shows both plotted points of intensity and a superimposed Gaussian curve which was normalized to the experimental data using the triangle method (referenced in methods). The multipliers (sensitivity) calculated from the triangular method were 2293192, 2778374, 2695154 for the 400, 700 and 1000 m/s, respectively, on panel B.

DISCUSSION.

Figure 2 demonstrates that the experimental resolving power plots match well with the simulated plots. The maxima and the minima in both sets of data gen-

erally align and qualitatively the data forms a general parabolic shape in both plots. This shows that, qualitatively, the model is accurate in predicting the settings that elicit the highest resolving powers. As expected, in most of the cases the experimental values did not reach the theoretical values due to inefficiencies afforded by real world conditions.

The cocaine resolving power plot (Figure 2D) appears anomalous due to its experimental RP being greater than the theoretical RP. Due to the instrumental configuration, after the TWIMS separation the ions pass through ion optics and into a time-of-flight (TOF) mass spectrometer before the drift time value is measured on the detector, which increases the measured drift time. Due to this increase in drift time, the numerator component of resolving power (t) may have been slightly enlarged. This anomaly doesn't appear to propagate as intensely in the other plots, because cocaine is almost twice as heavy as MDMA and three times as heavy as amphetamine, thus there is a smaller t contribution for the two lighter ions. As this study attempts to find the settings at which resolving power would be highest, it was not a priority to correct these values.

As expected the lower gate widths resulted in higher RP because gate width, by definition, is the length of time in which ions are permitted to enter the ion mobility spectrometer. Longer gate widths permit more ions to be measured, but also broadens the peak width and subsequently lowers the resolving power. Of great interest are the local maxima of the resolving power plots as these are the settings that offer the most resolution. By observing the plots (Figure 2) there appear to be distinct maxima in each profile and then the plot begins to fall again as resolving power falls. This indicates that there is an optimal setting to attain the highest resolution.

Due to computational limits, each point in the plot of the theory (Figure 2A, B, and C) summarizes the trajectories of 3003 ions (1001 ions per run which were run in triplicate; each run taking *ca.* 6 hours to complete). The experimental panels (Figure 2D, E, and F) summarize *ca.* 10^5 to 10^7 ions per point with ion counts (sensitivity) fluctuating based on the wave velocity.

For the theoretical results, RP rises abruptly at low wave velocities, while experimental results do not show this behavior. One possible explanation is that at lower wave velocities, the experimental peaks are no longer discreetly resolved from the other impurities in the sample, and thus the peak widths represent a composite of ion signals, making them broader than the theory where only a single ion species is simulated.

CONCLUSION

Qualitatively, the resolving power plots and the ion intensity plots show similarities in trend, with the general shape of both curves aligning, as well as the relative intensities of the drift spectra showing a similar profile. Some discrepancy between theory and experiment is expected as the theoretical model does not account for pressure or temperature gradients, charge repulsion, or local differences in the electric field that occur in the real world. While there are differences between the simulation and experimental data, the simulation is still valid for usage as the peak in RP curves offset is small enough for a researcher to determine optimal settings. The use of the simulation greatly reduces a researchers time in finding the optimal setting.

Future Directions

Although this study addressed changes in many different settings, different drift gas pressures were not evaluated. Some experiments were performed to test resolution at a lower pressure, the sensitivity was so low that data was not accessible from these experiments [4]. Another parameter that is worth investigating is the drift gas composition. Changing the nitrogen gas to a more polar gas (such as carbon dioxide) or a larger, monatomic gas (such as argon) may benefit the separation of certain analyte systems. To do this, a custom gas manifold would need to be constructed.

While other studies have been performed to affirm the validity of the predictive capabilities of SIMION, it is important to test the validity of this specific instrumentation geometry when used alongside the TWIMS-specific geometry and

user program [6]. The tandem use of the user created program and SIMION is novel and thus its accuracy, prior to this study, has not been tested. Thus these programs accuracy must be ensured before widespread implementation. A similar study with a different commercial TWIMS instrument can validate whether or not the SIMION program is accurate at generating the proper geometries and collision conditions. It would be interesting to determine if other resolving power trends can be predicted with different instrumentation setups and whether similarities are found between the theory and experimental results for the other instrumental geometry, as were found for the Synapt G2 experimental and instrumental configurations utilized in this study. Furthermore, it would be insightful to compare the two plots quantitatively and would be the basis of a future study.

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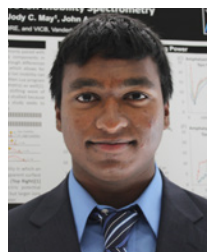
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