

AN IMPROVED CALIBRATION APPROACH FOR TRAVELLING WAVE ION MOBILITY SPECTROMETRY: ROBUST, HIGH-PRECISION COLLISION CROSS SECTIONS

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OVERVIEW

PURPOSE: Development of significantly improved methods for calibration of travelling wave ion mobility (TWIM) spectrometry data

METHODS: Updated TWIM theory and ion optical simulations. Calibration of experimental data for a wide range of compounds measured under a broad range of experimental conditions on two instrument platforms

RESULTS: CCS calibrations of significantly improved precision and accuracy across all conditions

INTRODUCTION

Travelling Wave Ion Mobility, since its introduction in 2004, has developed into a ubiquitous separation tool, impacting global efforts in areas ranging from food analysis to drug discovery. TWIM separates ions using a series of DC waves operating in a gas-filled ion guide. The relationship between the average ion velocity achieved and the TWIM operating parameters remains incompletely understood, so TWIM instruments are typically calibrated to obtain accurate collision cross section (CCS) values. Measurement of TWIM CCSs for large, multiply-charged species can be particularly exacting and requires careful choice of calibrant and expert evaluation of the resulting calibration curves. Here, we introduce a dramatically improved calibration methodology, yielding robust calibrations over a significantly improved range of ions and conditions.

METHODS

Developments in TWIM Theory

We have reported previously^{1,2} that, when expressed in suitable dimensionless coordinates, the motion of an ion in a one dimensional, smoothly moving sinusoidal travelling wave device with constant wave velocity and height can be modelled using the following differential equation of motion

$$\alpha \frac{d^2 z}{d\tau^2} = -\frac{dz}{d\tau} - 1 + \gamma \sin z \quad (1)$$

$$\text{where } \gamma = 2\pi \frac{V_0}{v\lambda} K, \quad \alpha = 2\pi \frac{v}{\lambda} \frac{K m}{q}$$

K is the ion mobility, V_0 is the on-axis wave amplitude, v is the wave velocity, λ is the wavelength and m/q is the mass-to-charge ratio of the ion. When $\alpha > 0$ this model includes velocity relaxation effects that are important for large molecules such as proteins, and the measured cell transit time depends both on mobility and mass-to-charge ratio. For small α it can be shown (extending previous results¹) using perturbation theory that the resulting average ion velocity in the laboratory frame of reference is, to order α^2 ,

$$\bar{v}_{\alpha,6} = v(1-\omega_0) \left[1 - \alpha^2 \omega_0^2 - \alpha^4 \omega_0^2 (2 + 3\omega_0 - 6\omega_0^2) - \frac{1}{8} \alpha^6 \omega_0^2 (49 + 81\omega_0 - 205\omega_0^2 - 341\omega_0^3 + 424\omega_0^4) \right] \quad (2)$$

where $\omega_0 = \sqrt{1 - \gamma^2}$. This expression is valid for all $\gamma < 1$.

Similarly, to order γ^6 we have the new result obtained by solving (1) perturbatively using the Lindstedt-Poincaré technique

$$\bar{v}_{\gamma,6} = \frac{v}{1 + \alpha^2} \left[\frac{\gamma^2}{2} + \frac{\gamma^4}{8} \frac{1 + 10\alpha^2 + 15\alpha^4}{(1 + \alpha^2)^2 (1 + 4\alpha^2)} + \frac{\gamma^6}{16} \frac{1 + 23\alpha^2 + 234\alpha^4 + 1171\alpha^6 + 2291\alpha^8 + 1620\alpha^{10}}{(1 + \alpha^2)^4 (1 + 4\alpha^2)^2 (1 + 9\alpha^2)} \right] \quad (3)$$

The results (2) and (3) can be combined into a single expression that gives average ion velocities which agree with numerical solutions of (1) to within 0.5% for all combinations of α and γ routinely encountered in practically useful TWIM experiments:

$$\bar{v}_{\text{blend}} = \frac{\gamma^{12}}{\gamma^{12} + \alpha^8} \bar{v}_{\alpha,6} + \frac{\alpha^8}{\gamma^{12} + \alpha^8} \bar{v}_{\gamma,6} \quad (4)$$

Real TWIM experiments differ from those modelled by (1) in several important ways: the waveform on the optical axis is not perfectly sinusoidal; waves progress in discrete steps rather than moving smoothly along the device; ions are not confined to the optical axis (off-axis ions experience higher wave voltages than those on-axis); the pressure and temperature inside the device may not be known accurately. Despite improved understanding of TWIM, calibration of these devices is therefore required. To assist in the investigation of these effects, a SIMION³ model has been created that includes discrete stepping of the travelling wave, an anharmonic and radially dependent TW field, off-axis motion with RF confinement and diffusion using the SDS collision model.

Calibration Methods

Historically, TWIM calibration has been carried out using power law expressions⁴ that are essentially of the form

$$\bar{v}_{\text{ion}} = a K^b \quad (5)$$

While these fits perform well for singly charged small molecule calibrations and large molecule calibrations under carefully chosen (low velocity) travelling wave conditions⁵, a pure mobility calibration cannot model velocity relaxation effects. In earlier work^{1,2} we investigated a six-parameter calibration based on an expansion of (2) in powers of K

$$\bar{v}_{\text{ion}} = c_2 K^2 + c_4 K^4 + c_6 K^6 + c_{12} K^4 \left(\frac{m}{q}\right)^2 + c_{62} K^6 \left(\frac{m}{q}\right)^2 + c_{64} K^6 \left(\frac{m}{q}\right)^4 \quad (6)$$

and this form, which can accommodate velocity relaxation effects, gave good fits to calibration data for a wide range of species over a broad range of conditions. However the use of four additional parameters potentially makes this calibration susceptible to overfitting errors.

In this poster, we introduce and evaluate a novel third approach to TWIM calibration which directly makes use of the analytical approximation (4) to the solution of the equation of motion (1). In this approach, nominal values of α and γ are calculated for each ion for the selected travelling wave conditions, and these values are then rescaled by dimensionless calibration parameters a and g respectively. Both a and g are expected to have values close to 1.0 and it is sometimes convenient to fix $a=1.0$ to further reduce the number of free parameters

$$\bar{v}_{\text{ion}} = \bar{v}_{\text{blend}}(g, a) \quad (7)$$

Velocity and voltage ramping can be accommodated in this approach without additional parameters by correctly accounting for the variation of α and γ during the ramp.

Modification for Radial Effects

Realistic models of TWIM experiments show that ions of different species have different radial distributions and therefore experience slightly different axial fields. This effect is particularly apparent for small ions of different charge states. Figure 1 shows the mean radial position adopted by polyaniline ions under typical conditions on a Waters Synapt G2-Si instrument as well as the peak axial field at different radii.

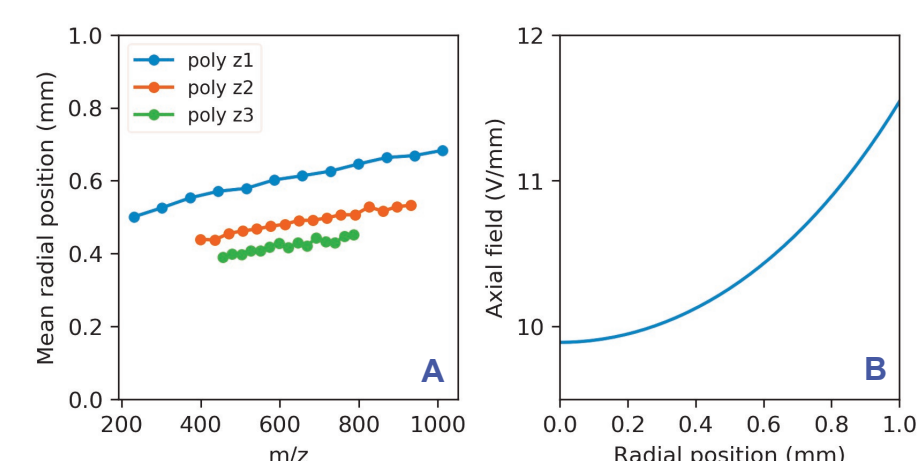


Figure 1 A) The mean radial positions occupied by singly, doubly and triply charged polyaniline ions in a simulation of the TWIM geometry utilised in the Waters Synapt G2 instrument at a pressure of 3mbar, an applied wave height of 20V and a wave speed of 800m/s. B) Peak axial field as a function of radial position.

In this poster, we evaluate a modification of the three calibration expressions (5), (6), and (7) obtained by multiplying the average ion velocity by a correction factor of the form

$$e^{c/\sqrt{z}} \quad (8)$$

where c is an additional undetermined calibration parameter.

Experimental

Waters Synapt G2: Native proteins cytochrome C (Cyt C), b-lactoglobulin, avidin, bovine serum albumin (BSA), transthyretin, concanavalin, alcohol dehydrogenase (ADH), pyruvate kinase, glutamate dehydrogenase; denatured proteins ubiquitin and Cyt C; and polyaniline were prepared as specified previously². Lipids POPC, Egg SM, DOPE and DMPC in chloroform were diluted in 90% chloroform / 9.9% methanol / 0.1% formic acid to a final concentration of 10 $\mu\text{g}/\text{ml}$.

Arrival time distributions were recorded over a broad range of travelling wave amplitudes and velocities at a pressure of ~ 3.5 mbar N_2 in the TWIM cell.

Waters SELECT SERIES Cyclic IMS: A single-pass calibration study was also performed on a Waters cyclic ion mobility-enabled quadrupole time-of-flight mass spectrometer⁶. The CIM device consists of a 98 cm path length RF ion guide comprising over 600 electrodes around which T-Waves circulate to provide mobility separation in ~ 2 mbar N_2 . The calibration set consisted of Major Mix (Waters Corporation) as well as the native proteins Cyt C, BSA and ADH.

Data processing and calibration was carried out using custom scripts. Reference CCS values were obtained from published drift tube measurements^{7,8,9}.

RESULTS

All-Ion Calibrations

The heat maps in Figure 2 (B-D) show the percentage root mean square errors (%RMSE) for all-ion calibrations obtained using (5), (6) and (7) respectively and (E-G) show the corresponding results with the inclusion of a radial term of the form (8). The heat maps B) and E) corresponding to power-law calibrations (5) with and without radial corrections confirm that with the traditional approach, high quality all-ion calibrations are only achieved at low wave velocities as originally reported by Zhong *et al.*⁵

The remaining four heat maps C), D), F) and G) correspond to calibration functions (6) and (7) which incorporate velocity relaxation effects. These plots all demonstrate a clear and significant improvement over the power-law results. In particular, there is little evidence of any preference for low wave velocities suggesting that relaxation effects have been largely absorbed by the calibration.

In the all-ion results there is a general, but very modest, preference for the forms incorporating radial effects (at the cost of introducing an additional calibration parameter).

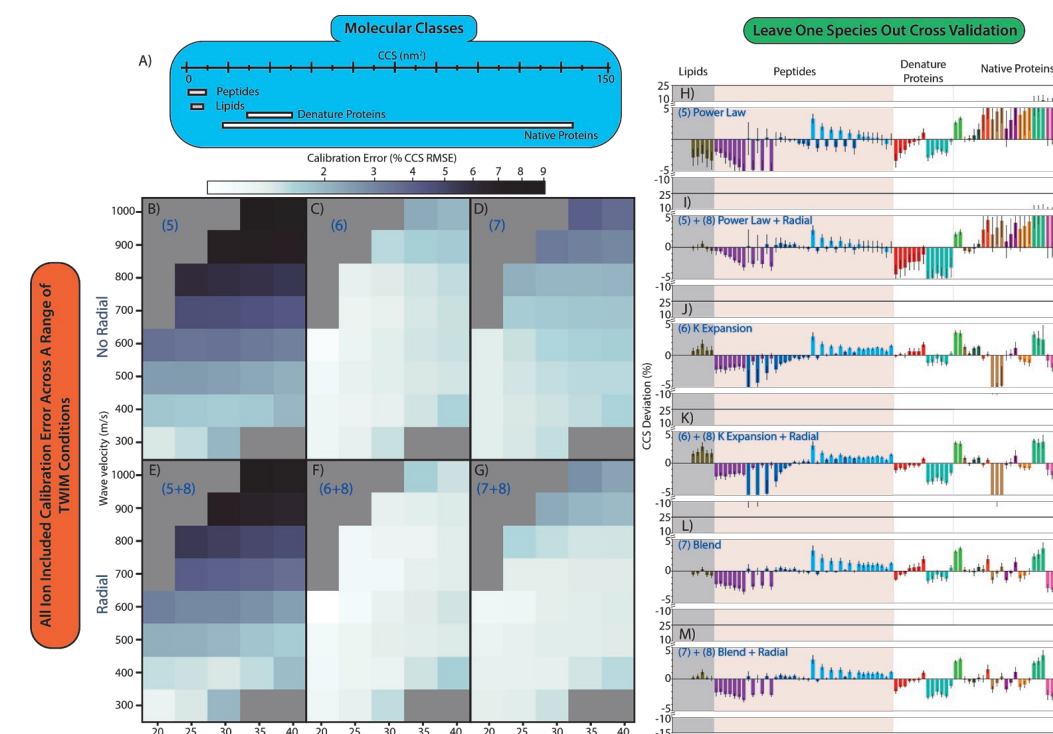


Figure 2. Performance of the calibration methods over a matrix of TWIM conditions for all ions. No wave ramping was performed. A) The range of CCS values for each of the included molecular classes. B), C), D) heat maps showing the dependence of %RMSE calibration error on TWIM conditions using Eq. (5), (6), (7) respectively. E), F), G) corresponding results when a radial correction of the form (8) is included. H-M) leave one species out cross-validation results for CCS deviation. The solid bars are averages over all TWIM conditions and the error bars show the standard deviation of the results.

In the cross-validation results it can be seen that incorporation of velocity relaxation in the calibration, (J)-M), generally results in a substantial reduction in the size and variability of the protein residuals. There is, however, evidence of overfitting in the six-parameter power law results (J) and K) which is manifested through large residuals for native avidin (brown) and 2⁺ polyaniline (blue). In contrast, the results L) and M) obtained using the blended form using only two and three parameters respectively are much more robust. In what follows, we therefore discard the K expansions and focus on the power law and blend calibrations.

Blend+radial calibrations were also carried out on the Synapt G2 instrument under velocity ramped TWIM conditions (30V, 1000-600m/s). CCS %RMSE for all-ion calibrations were 1.3% or below.

All-ion blend+radial calibration results for a limited set of species obtained at three different wave heights using the Waters Cyclic IMS instrument are shown in Figure 3. Although the TWIM electrode geometry is significantly different in this device, the results are of very similar quality to the Synapt data shown in Figure 2.

Calibration Using Restricted Sets of Ions

Although demonstration of all-ion calibrations is an important validation of the approach taken in this work, most real-world applications are concerned with a particular molecular class (for example small singly charged molecules or native proteins). Determination of the optimal calibration methodology for restricted classes is therefore of particular interest. Figure 4 contrasts %RMSE and CCS deviations for calibrations created using individual molecule classes with an all-ion calibration.

The sample preparation and experiment time needed to produce the all-ion calibration data makes its routine use impractical. We have therefore explored possible minimal calibration sets with a view to designing a single simplified sample that calibrates for as wide a range of species as possible. Figure 5 shows results for one such calibration set consisting of only six ions: the 14⁺-16⁺ charge states of native BSA and the (Ala)₇-(Ala)₉ peptides. This particular set is of interest because it should be possible to prepare and spray a mixture of native BSA and some suitable peptides (other than polyaniline) in a single solution.

The CCS of the 68⁺ charge state of the 14mer of the GroEl protein was measured using this minimal calibration, giving a value 3.6% above the published value¹⁰ compared with +15.5% for a power-law calibration. The extrapolation from $\sim 45\text{nm}^2$ (BSA) to $\sim 220\text{nm}^2$ illustrates the robustness of the calibration.

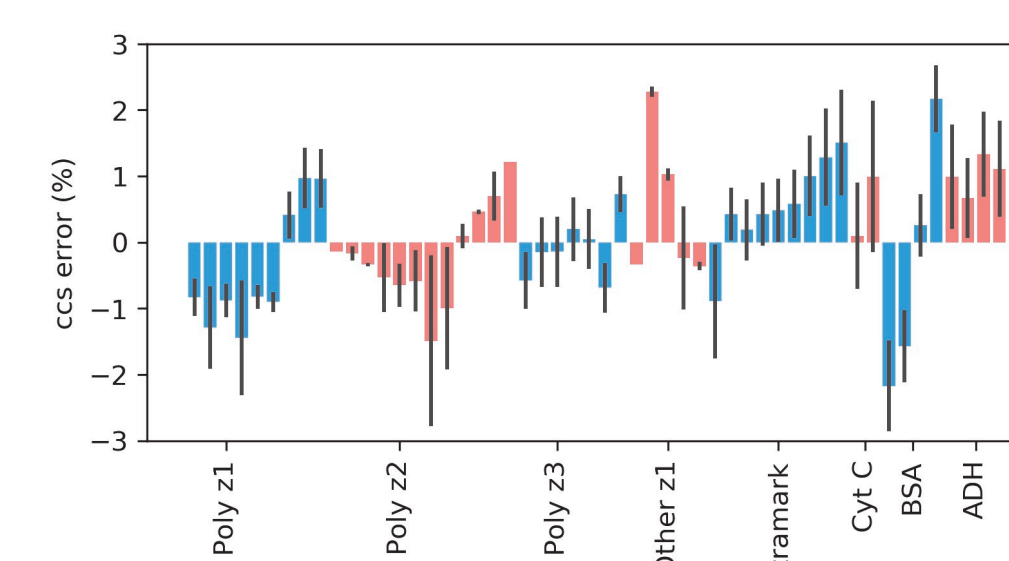


Figure 3. Blend+radial calibration results ($a=1.0$) for Cyclic IMS data acquired at wave heights of 30, 40, 50V and wave velocity 375 m/s.

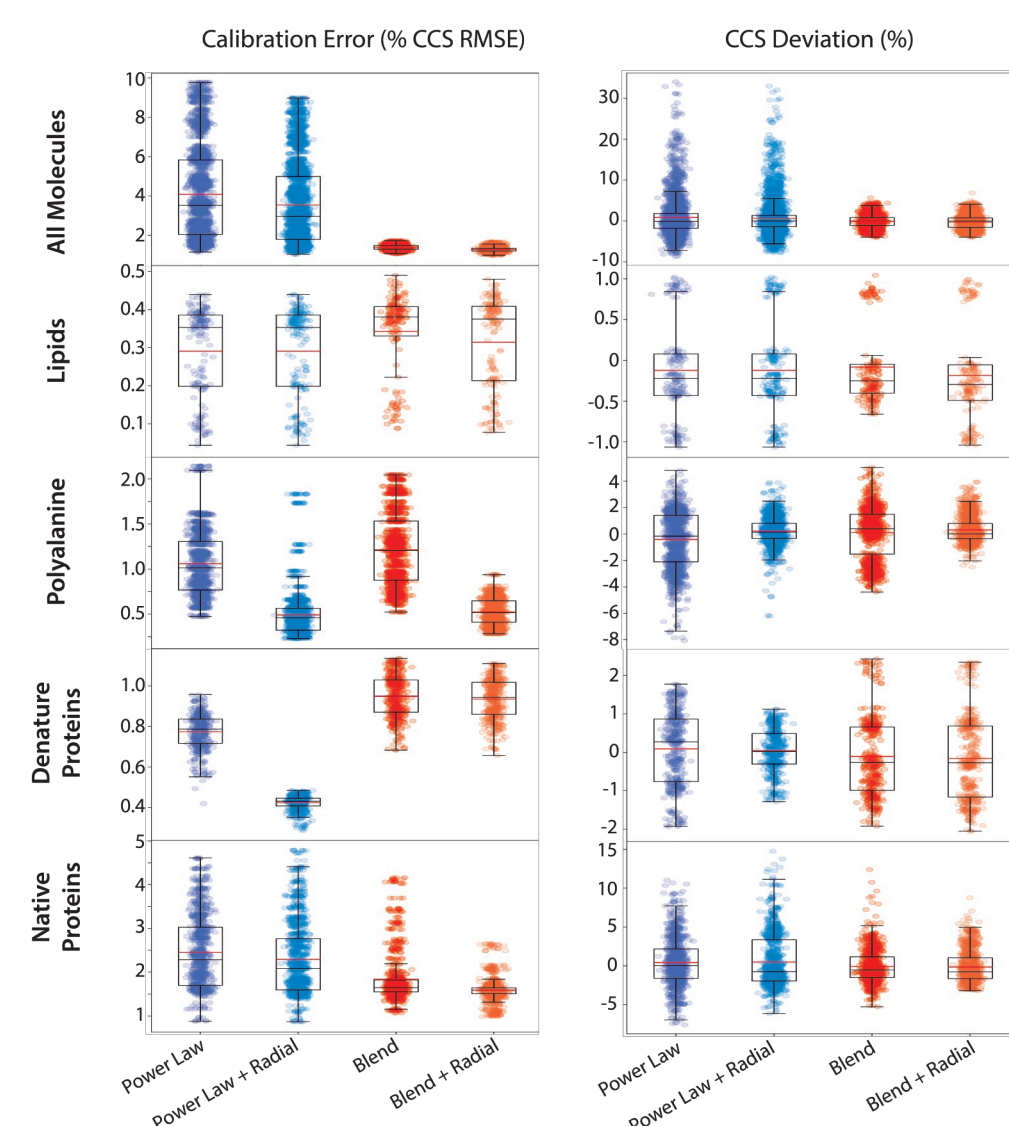


Figure 4. Comparison of all-molecule and single molecule class calibrations produced using power law and blend functions with and without the radial correction. In the blend calibrations, the parameter a was fixed at its natural value of 1.0 so that the blend and blend+radial calibrations utilised only one and two parameters respectively.

DISCUSSION

The TWIM calibration function comprised of the blend (7) and exponential radial function (8) performs robustly in all-ion calibrations on both instrument platforms and restricted class calibrations, and significantly better than power-law calibrations for large and multiply charged molecules. It can accommodate the velocity relaxation effects exhibited by large molecules, as well as the subtle radial effects exhibited by small molecules with low charge states. The approach is also resistant to overfitting as demonstrated through cross-validation of the results and extrapolation of a minimal ion calibration.

We show all-ion calibrations with %RMSE of around 1% while the peptide and lipid only calibrations have %RMSE which are consistently sub 1%. Small molecule applications may therefore still benefit from dedicated small molecule calibration sets.

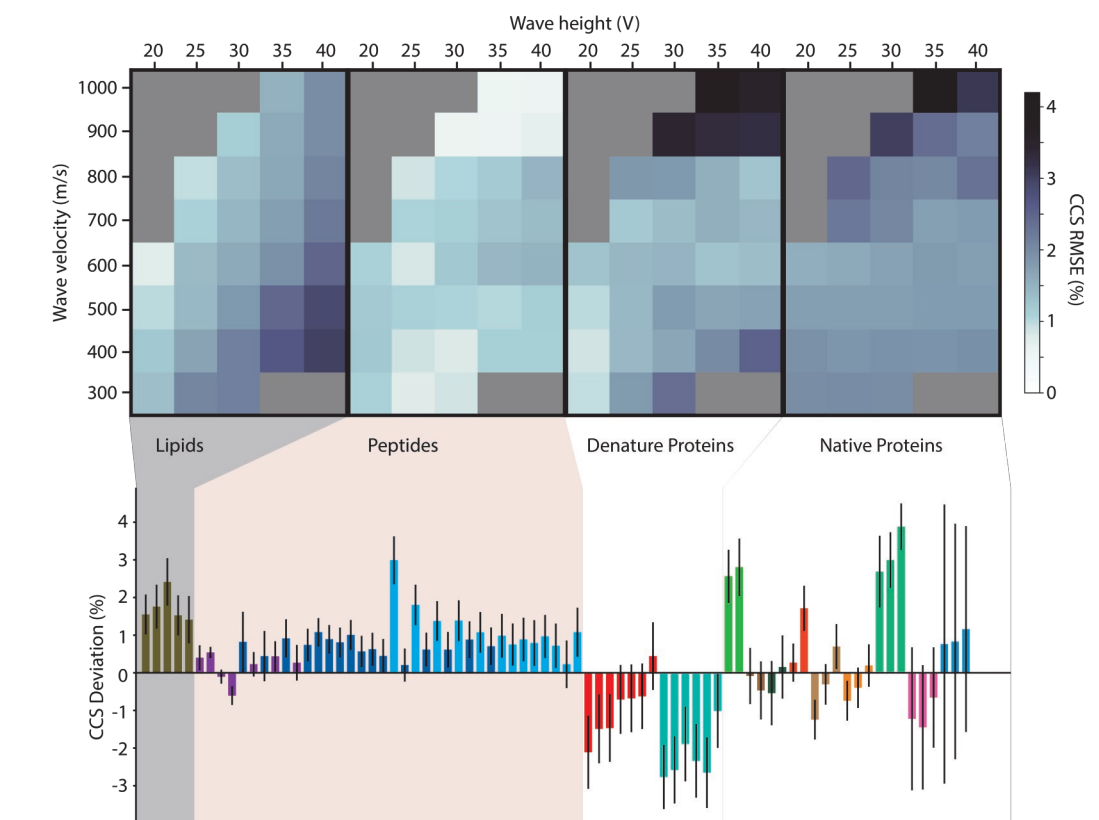


Figure 5. Blend+radial calibrations ($a=1.0$) created using the minimal calibration set consisting of 14⁺-16⁺ charge states of native BSA and the (Ala)₇-(Ala)₉ peptides. Top: CCS %RMSE for different TWIM conditions. Bottom: CCS deviations for individual ions.

We have shown that a simple mixture containing a single native protein and a few small peptides is sufficient to produce high quality results across an extremely wide CCS range. Future work will include development and testing of such a sample.

We have also demonstrated that the approach outlined here can be used successfully with a new Cyclic IMS geometry as well in experiments that employ wave velocity ramping.

The accuracies of the calibrations produced here are comparable with the reported accuracies of some of the reference compound measurements and analysis of the residuals suggests that further improvements may require higher precision drift tube measurements. These will also be required for calibration of future high resolution IMS experiments.

CONCLUSION

- We introduce new TWIM CCS calibration functions that allow significantly improved precision and accuracy over a wide range of conditions, molecule types and CCS values
- We show robust and high quality all-ion calibrations utilising only two and three free parameters
- Results are presented for linear and cyclic TWIM geometries under static and ramped travelling wave conditions
- We show the performance of a minimal calibration set and discuss the design of a similar set which could be prepared as a single mixture
- The results highlight the need for higher precision CCS measurements of robust calibrants

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