Calibration of Charge Detection MS Instruments

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OVERVIEW

PURPOSE:

- Introduce *m/z* and charge calibration of a charge detection MS (CDMS) instrument.
- Develop a novel approach to *m/z* calibration using high-mass standards having uncertain masses.
- Quantify and propagate uncertainties in *m/z* following calibration.

METHOD:

- *m/z* calibration using a single parameter and two-parameter linear calibration of charge state.
- Specify a theoretical minimum mass for each standard.
- Adopt a Bayesian approach that explicitly acknowledges the uncertainty in the additional mass.

RESULTS:

- We obtain *m/z* calibrations having precision better than 0.1% and charge precisions of significantly less than unit charge for measurements over 100 ms trapping times.

INTRODUCTION

Charge Detection Mass Spectrometry (CDMS) has the potential to significantly extend the utility of mass spectrometry for the analysis of high molecular weight and highly heterogeneous samples. This is possible because simultaneous measurement of the mass-to-charge ratio (*m/z*) and charge state (*z*) of individual ions allows determination their mass, significantly reducing the complexity of electrospray MS data for these species.

We describe approaches to both *m/z* and *z* calibration, using appropriate standards and a novel algorithmic treatment in order to capture and propagate relevant uncertainties.

An unusual aspect of CDMS calibration is that, owing to their size, calibration standards can carry additional mass (e.g. residual solvent), and we explain how this can be accounted for during the calibration process.

METHODS

The Electrostatic Linear Ion Trap CDMS (ELIT CDMS) instrument used in this study is shown in **Figure 1**. Oscillating ions in the trap induce a charge on the detector tube which is measured using a charge-sensitive amplifier. The design of the detection tube ensures that 100% of the ions' charge is is induced onto the detector, allowing high-resolution charge measurements for each individual ion.

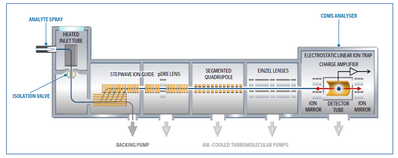

Figure 1. The Electrostatic Linear Ion Trap Charge Detection Mass Spectrometer

Indeed for sufficiently long trapping times, the charge of individual ions can be determined exactly[1]. In order to do this, however, a calibration relationship must be established between the magnitude of the observed signal and the charge state of the ions.

Careful amplifier design and appropriate signal processing ensures that the magnitude *A* of the induced voltage is nearly proportional to each ion's charge state. In practice a linear calibration is employed to allow for (and measure) small offsets in charge measurement:

$$z = c_1 + c_2 A \qquad (1)$$

These constants are determined using protein standards that can be resolved by *m/z* in order that a representative magnitude can be determined for each charge state.

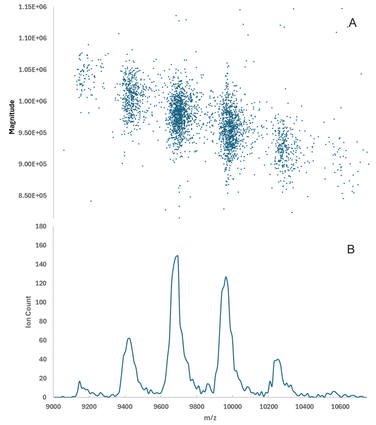

Figure 2. CDMS data corresponding to six charge states of the hexamer of L-Glutamate Dehydrogenase (GDH). A) Magnitude plotted against m/z ratio. Each dot represents a 100 ms trapping time single ion detection. B) The same data represented as a conventional m/z spectrum with a provisional calibration.

Figure 2A shows a scatter plot of individual ion detections for a protein standard with the *y*-axis representing signal magnitude *A*. Although the 100 ms trapping time employed here (charge RMSD ~0.9e) does not result in baseline charge resolution (~0.2e), the gradual decrease in magnitude with *m/z* (decreasing charge state) is visible.

Because the charge states are resolved by m/z, a charge calibration can formed by determining representative magnitudes corresponding to individual charge state peaks for a number of standards. Having done this, the parameters c_1 and c_2 in equation (1) can be determined using a straightforward linear fit. However in order to do this it is necessary to correctly assign charge states to these peaks, which requires *m/z* calibration.

M/Z CALIBRATION

It is desirable to utilize calibration standards having a wide range of charge states to establish a high-quality charge calibration. The same standards can also contribute usefully to *m/z* calibrations. However higher mass standards can also carry a significant amount of extra mass in the form of adducts. This uncertain additional mass must be taken into account during *m/z* calibration.

The relationship between frequency and *m/z* in an electrostatic ion trap is given by

$$\frac{m}{z} = \frac{k}{F^2}$$

Where *k* is a constant (to be calibrated) which depends on the geometry of the trap, ion energy, and the applied voltages. The calibration dataset will typically consist of series of data for *N* standards having known masses $m_1, m_2, \ldots m_N$. Each of these standards will be represented in the data by a number of charge states $z=z_{n1}, z_{n2}, \ldots$ for the *i*'th standard (*1≤i≤N*). We allow that each standard has some *a-priori* unknown amount of excess mass δ_i attached which we currently assume to be the same for each charge state so that the observed *m/z* values d_{ij} (where j indexes charge states), obtained using a provisional calibration will be

$$d_{ij} \sim g\frac{m_i + \delta_i}{z_{ij}} \pm \sigma_{ij}$$

where *g* is an *a-priori* unknown scaling factor (*g=1.0* if the provisional calibration constant *k* is exactly correct). Following calibration, *k* will be updated to a revised value *k'=k/g*. The σ_{ij} are uncertainties in the *m/z* measurements which are obtained by peak detecting a spectrum such as that shown in ***Figure 2B***. Note that for simplicity we have ignored the mass of charge carriers here: although relatively small these are easily included in the analysis.

We adopt a Bayesian approach, which requires that we assign prior probability distributions to the unknown parameters *g* and δ_i. To avoid unnecessary complication here we assign an improper uniform prior to the calibration parameter *g*. Because the excess mass parameters δ_i are known to be positive, an exponential prior for these is appropriate:

$$\Pr(\delta_i) = \frac{1}{\Lambda_i}\exp\left(-\frac{\delta_i}{\Lambda_i}\right)$$

where Λ_i is the expected scale of additional mass (e.g. 1% of m_i). Assuming a Gaussian likelihood function, the joint probability of the data and parameters is

$$\Pr(d,\delta_1,\delta_2,\ldots,g) = \prod_i \frac{1}{\Lambda_i}\exp\left(-\frac{\delta_i}{\Lambda_i}\right)\prod_{j\in z_i}\frac{1}{\sqrt{2\pi}\sigma_{ij}}\exp\left(-\frac{\left(d_{ij}-g\frac{m_i+\delta_i}{z_{ij}}\right)^2}{2\sigma_{ij}^2}\right)$$

$$= \frac{1}{(2\pi)^{N/2}}\prod_{ij}\frac{1}{\sigma_{ij}}\prod_i\frac{1}{\Lambda_i}\exp\left(-\frac{(\delta_i-\delta_{i0})^2}{2S_i^2}\right)\exp\left(\sum_i\left(\frac{B_i^2}{4A_i}-C_i\right)\right)$$

where in the second line we have simply rearranged the expression (to facilitate marginalization) and defined the new symbols

$$A_i = \frac{1}{2}g^2X_i \qquad B_i = g^2m_iX_i - gY_i + \frac{1}{\Lambda_i}$$

$$C_i = \frac{1}{2}g^2m_i^2X_i - gm_iY_i + \frac{1}{2}Z_i \qquad S_i = \frac{1}{\sqrt{2A_i}} \qquad \delta_{i0} = -\frac{B_i}{2A_i}$$

$$X_i = \sum_{j\in z_i}\frac{1}{z_j^2\sigma_{ij}^2} \qquad Y_i = \sum_{j\in z_i}\frac{d_{ij}}{z_j\sigma_{ij}^2} \qquad Z_i = \sum_{j\in z_i}\frac{d_{ij}^2}{\sigma_{ij}^2}$$

Since we are not primarily interested in the additional mass parameters δ_i, they can be "marginalized" i.e. integrated out of the full joint probability to give the joint probability of *g* and the data:

$$\Pr(d,g) = \int_0^\infty\int_0^\infty \Pr(d,\delta_1,\delta_2,\ldots,g)\,\mathrm{d}\delta_1\mathrm{d}\delta_2\ldots$$

$$\propto \exp\left(\sum_i\left(\frac{B_i^2}{4A_i}-C_i\right)\right)\prod_i S_i\left(1+\mathrm{erf}\left(\frac{\delta_{i0}}{\sqrt{2}S_i}\right)\right) \qquad (2)$$

where erf is the error function. Everything in this joint probability distribution is known except *g*, so despite the apparent complexity it is just a 1D distribution. It is therefore straightforward to obtain samples of *g* to determine the required calibration scaling *g* together with the associated uncertainty. The posterior probability distribution Pr(*g*|*d*)=Pr(*g*,*d*)/Pr(*d*) is obtained by normalizing with respect to g if required. The denominator Pr(*d*) is the "Evidence", obtained by integrating Pr(*g*,*d*) over *g* and is useful in model comparison (e.g. comparing possible charge state assignments).

We illustrate this new calibration approach using simulated data for three hypothetical high-mass calibrants as described in ***Table 1***.

Calibrant #	1	2	3
Expected Mass (kDa)	150	350	600
Min Charge State	20	40	60
Max Charge State	24	45	63
Expected Excess Mass (kDa)	0.15	3.5	6.0
Simulated Mass (kDa)	150.04	353.41	608.89

Table 1. Expected masses, charge state ranges, expected excess mass and simulated masses for the three simulated calibrant species.

Simulated mass excesses for each calibrant were sampled from exponential distributions having scale factors set by the expected excess masses (Λ_i) shown in the table. Simulated *m/z* values d_{ij} for each charge state were then sampled from Gaussian distributions with a standard deviation of 100ppm. The simulated calibration scaling factor was g_t=*1.1*.

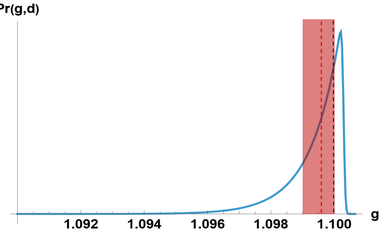

Figure 3. Probability distribution (blue) for the calibration scale parameter g and the data. The dotted red line shows the position of the median: g_m=1.0996 and the shaded region is the interquartile range (Q1,Q3) =(1.0990,1.1001). The black dotted line shows the true value g_t=1.1.

The resulting joint probability distribution Pr(*g*,*d*) is shown in **Figure 3**. The most obvious feature of this is the sharply asymmetrical distribution. This correctly reflects the fact that *g* cannot take a value that would imply a negative value for any of the δ_i since these are mass *excesses*. Because of this asymmetry, we choose to report a median and interquartile range rather than a mean and standard deviation for the distribution. The median is g_m=1.0996, which is 0.03% below the true value g_t=*1.1*, and the true value lies within the interquartile range.

EXPERIMENTAL RESULTS

L-Glutamate Dehydrogenase (GDH), human serum albumin (HSA), Enolase, beta-lactoglobulin (BLG) and the Waters™ mass check mAb standard were buffer exchanged into 200 mM ammonium acetate solution using Bio-Spin® P-6 size-exclusion columns (Bio-Rad Laboratories). Ions were generated in positive ion mode using nanoelectrospray ionization and mass analysis was performed using two mechanically equivalent prototype electrostatic linear ion trap CDMS systems CDMS1 and CDMS2. At the time the calibration of the CDMS2 instrument was made, the BLG sample was not available. Signal processing and data visualization were performed using novel software developed in-house. Ions were trapped for 100ms and the frequency and amplitude information were converted to *m/z* and *z* values respectively, and ultimately mass (*m/z* * *z*). Spectra were peak detected using a modified deconvolution algorithm to obtain an *m/z* value for each charge state of each standard. The details for each calibrant are shown in ***Table 2***.

Calibrant	GDH	HSA	Enolase	BLG*	mAb
Expected Mass (kDa)	333.5	66.4	93.37	18.36	149.1
Min Charge State	34	15	18	6	21
Max Charge State	39	17	20	7	24
Expected Excess (kDa)	7	1	1	0.02	0.1

*Table 2. Expected masses, charge state ranges, expected excess masses and simulated masses for the experimental calibrant species. *BLG was not used in the calibration of the CDMS2 instrument.*

The calibration results for the two instruments are shown in ***Table 3***.

Instrument	CDMS1	CDMS2
Scaling Factor (g)	1.0314	1.0348
Interquartile Range (ppm)	330	541
RMS m/z error (ppm)	593	648

Table 3. Inferred scale factors for instruments CDMS1 and CDMS2 with associated interquartile ranges and RMS (residual) errors expressed in parts-per-million (ppm).

Prior to calibration, the instruments had identical provisional calibrations, so it is notable that the scaling factors lie within 0.4% of each other, suggesting good reproducibility in instrument construction. The detailed *m/z* residuals for the CDMS1 instrument are shown in ***Figure 4***.

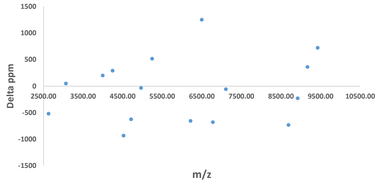

Figure 4. m/z residuals for calibrant peaks following calibration of the CDMS1 instrument. The RMS m/z error is 593 ppm.

Following m/z calibration, representative magnitudes were extracted for each charge state, allowing construction of a charge state calibration. The resulting linear calibration (see Eq. (1)) and associated residuals for the CDMS2 instrument are shown in ***Figure 5***. The RMS charge residual is 0.093 elementary charges, demonstrating excellent linearity of charge measurement over the range of the calibrants. The intercept is –0.62 charges, further indicating near proportionality.

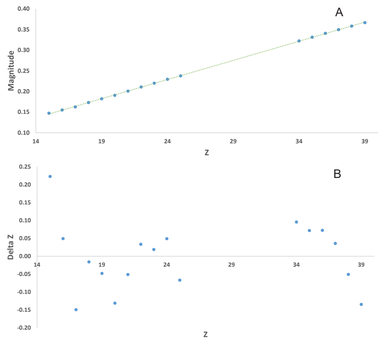

Figure 5. A) Linear charge calibration of the CDMS2 instrument. The charge offset is –0.62 elementary charges demonstrating near proportionality. ***B)*** Charge calibration residuals. The RMS residual is 0.093 elementary charges.

DISCUSSION

We have demonstrated a novel and robust approach to m/z calibration of MS data which is applicable when the masses of some calibrants are uncertain but bounded below by a minimum mass.

We achieve an m/z calibration of a novel Electrostatic Ion Trap CDMS instrument using five protein standards with an RMS of ~600ppm.

Calibration of two identical prototype instruments yielded calibrations that agree within 0.4% suggesting highly reproducible instrument construction.

Charge calibration of the instrument has also been demonstrated, showing near proportionality between number of charges and FFT magnitude and an RMS of less than 0.1 elementary charges.

Despite the availability of relatively mass-homogenous calibrants such as BLG and the mAb, explicitly modelling additional mass allows it to be monitored and controlled.

CONCLUSION

- **A robust probabilistic approach to CDMS calibration**
- **Reproducible calibration of two novel prototype CDMS instruments**

References

1. Todd, A. R. and Jarrold, M. F.. J. Am. Soc. Mass Spectrom. 2020, 31, 6, 1241–1248

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