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International Journal of Mass Spectrometry 265 (2007) 99–105

International Journal of Mass Spectrometry

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Experimental evidence for space-charge effects between ions of the same mass-to-charge in Fourier-transform ion cyclotron resonance mass spectrometry

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Received 4 November 2006; received in revised form 19 January 2007; accepted 23 January 2007
Available online 30 January 2007

Abstract

It is often stated that ions of the same mass-to-charge do not induce space-charge frequency shifts among themselves in an ion cyclotron resonance mass spectrometry measurement. Here, we demonstrate space-charge induced frequency shifts for ions of a single mass-to-charge. The monoisotopic atomic ion, Cs^+, was used for this study. The measured frequency is observed to decrease linearly with an increase in the number of ions, as has been reported previously for space-charge effects between ions of different mass-to-charge. The frequency shift between ions of the same *m*/*z* value are compared to that induced between ions of different *m*/*z* value, and is found to be 7.5 times smaller. Control experiments were performed to ensure that the observed space-charge effects are not artifacts of the measurement or of experimental design. The results can be rationalized by recognizing that the electric forces between ions in a magnetic field conform to the weak form of the Newton's third law, where the action and reaction forces do not cancel exactly.

Keywords: Mass accuracy; Accurate mass; Space-charge effect; FT-ICR; FTMS

1. Introduction

Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry, developed by Comisarow and Marshall [1,2], provides the highest mass accuracy [3,4] and highest mass resolution [5] of any mass analyzer. The ultrahigh mass accuracy provided by FT-ICR makes it possible to determine the elemental composition of small and large molecules from direct mass measurement [6–9]. Since the introduction of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MADLI), the demand for higher mass accuracy has increased with the growing complexity of the samples and of the average mass of the samples. The mass of ions is not directly measured in FT-ICR mass spectrometry. Rather, the cyclotron frequency of ions is measured and converted to mass using appropriate calibration equations [10–16]. The cyclotron frequency of an ion is known to decrease with increasing ion population in an analyzer ion cell, as a result of a space-charge induced frequency shift [10–13,15–18]. Therefore, mass accuracy in an externally calibrated spectrum is primarily limited by fluctuations of the ion population. McIver and coworkers [11] and Amster and coworkers [13,18] have demonstrated that mass accuracy can be substantially improved by correcting the frequency shift using a plot of frequency versus total ion intensity.

Wineland and Dehmelt have stated that ions of the same mass-to-charge cannot induce a space-charge shift in their own frequencies [19], and others have repeated this claim [20,21]. In recent years, however, the space-charge induced frequency shift has been shown to be different between ions of different *m*/*z* value than between ions of the same *m*/*z* value. Eyler and coworkers [14] and Smith and coworkers [15] have incorporated individual ion intensity as part of the calibration originally developed by Gross and coworkers [12]

$$\left(\frac{m}{z}\right)_{\mathrm{i}} = \frac{A}{f_{\mathrm{i}}} + \frac{B}{f_{\mathrm{i}}^2} + C\frac{I_{\mathrm{i}}}{f_{\mathrm{i}}^2} \tag{1}$$

where f_{i} is the measured cyclotron frequency of the calibrant ion at $(m/z)_{\mathrm{i}}$, I_{i} the corresponding ion intensity, and A, B, C are the fitting parameters. The first term on the right-hand side of Eq.

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doi:10.1016/j.ijms.2007.01.014

(1) expresses the effect of the magnetic field on the cyclotron frequency. The second term describes the frequency shift from the electric field of the applied trapping potential and from the space-charge of the ions, treating space-charge induce frequency shifts equally for all ions. The third term is a correction to the original equation developed by Gross and coworkers [12]. The third term "compensates" for differences between the space-charge for ions of the same *m*/*z* value versus those of different *m*/*z* value. The space-charge effects in the second and third term have also been referred as the global and local space-charge effects, respectively. The modified calibration equation (1) has shown to improve internal calibration mass accuracy by a factor of 1.5–6.7, depending on the mass range and the ion excitation radius [15]. Muddiman and Oberg [16] have demonstrated mass calibration improvement by including the local space-charge term in a global regression approach.

The global space-charge effects are significantly larger than local space-charge effects in multi-component spectra because individual peaks are small compared to the total intensity, and thus much of the space-charge induced frequency shift can be corrected without including the local space-charge term [11,13,18]. This explains why local space-charge effects have gone unnoticed by most FT-ICR practitioners. Although the local space-charge term is small in magnitude, it is a variable across any spectrum, making its significance far-reaching. Whereas the global space-charge term affects external calibration results and can be corrected for by using internal calibration, the local space-charge term impacts both external and internal calibration, and can only be corrected by using a more sophisticated calibration equation. Our group has recently developed a stepwise-external pseudo-internal calibration method [22], which has shown to improve mass accuracy 2–4 times. Most importantly, we have applied a new calibration

$$\left(\frac{m}{z}\right)_{\mathrm{i}} = \frac{A}{f_{\mathrm{i}} + B + CI_{\mathrm{i}}} \tag{2}$$

which is an extension of the calibration equation originally developed by McIver and coworkers [11]. f_{i} and I_{i} are the frequency and the intensity of the ion at $(m/z)_{\mathrm{i}}$, and A, B, C are the fitting parameters. The CI_{i} term is new to the original equation. The concept of including individual ion intensity I_{i} in Eq. (2) is similar to that of Eq. (1), and we have found a ∼2-fold additional mass accuracy improvement using Eq. (2) over the original equation [22].

In Eqs. (1) and (2), the global space-charge effects are constant for each mass spectrum and therefore are combined with the trapping potential shift as a single term. In contrast, Muddiman and Oberg [16] have demonstrated a global regression approach for polymers where the regression is performed on nine spectra simultaneously. Consequently, the global space-charge term is different for the nine spectra and can be separated from the trapping potential term. The resulting calibration equation is a three variable equation

$$\left(\frac{m}{z}\right)_{\mathrm{i}} = \frac{A}{f_{\mathrm{i}} + B + CI_{\mathrm{i}} + DI_{\mathrm{total}}} \tag{3}$$

where f_{i} and I_{i} are the corresponding frequency and ion intensity of the ion at $(m/z)_{\mathrm{i}}$, I_{total} the total ion intensity of the spectrum, and A, B, C, D are the fitting parameters. The cyclotron frequency is shown to be a function of the local space-charge term (CI_{i}). Surprisingly, the local space-charge parameter (C) is calculated to be 4 times that of the global space-charge parameter (D) and with an opposite sign. These results not only suggest that space-charge effects are more responsive to ions of the same *m*/*z* value than to other ions, but also that the effect is opposite in sign, that is, frequency increases with ion number.

The theoretical basis for separately treating ions of the same *m*/*z* value from ions of different *m*/*z* value is based on two publications by Wineland and Dehmelt on the measurement of electrons in a Penning trap [19,23]. In the first paper [23], Wineland and Dehmelt stated that the axial frequency of electrons parallel to the magnetic field (i.e., *z*-motion) is constant with respect to the number of electrons. The theoretical argument is that the forces between electrons are equal and opposite according to Newton's third law, and consequentially the center-of-mass motion of the electron cloud is the same as a single electron. Since the detector does not monitor motions of individual electrons, but rather the center-of-mass motion, the observed axial frequency is independent of the number of electrons. In the second paper [19], Wineland and Dehmelt extended the same argument for the motion perpendicular to the magnetic field, and predicted the center-of-mass motion in any direction is independent of the number of charged particles when these particles are of the same *m*/*z* value. In the FT-ICR mass spectrometry community, this statement is often repeated as "ions of the same *m*/*z* cannot space-charge frequency shift each other." Still, the theoretical prediction is made for a very ideal situation. Recent publications [15,16,22] have indicated that space-charge effects between ions of the same *m*/*z* value are different than those between ions of different *m*/*z* value, but none of these publications have demonstrated a zero space-charge contribution between ions of the same *m*/*z* value. In these studies, the frequency-to-mass conversions are shown to improve by including a linear order ion intensity term (CI_{i}) for multi-component mass spectra. A linear order correction is a reasonable approximation for short range correction because the first Taylor series expansion term for many functions are linear (e.g., $\sin(x)$, e^{x}, $1/(1-x)$, $\ln(1+x)$, ...). The real dependence may significantly deviate from linearity at long range. Consequently, the real space-charge effects for ions of the same *m*/*z* value cannot be extrapolated from multi-component spectra by using an approximate calibration equation. We are interested in the existence of space-charge effects between ions of the same *m*/*z* value because a better understanding of this effect will help advance the basic understanding of ICR ion motion, calibration equation development and the achievement of routine sub part-per-million (ppm) mass accuracy.

Here we present results from a direct measurements of space-charge induced frequency shift for an ion of one *m*/*z* value using the monoisotopic elemental ion, $^{133}\mathrm{Cs}^{+}$. The results demonstrate an existence of a space-charge induced frequency shift among ions of the same *m*/*z* value. The Cs^{+} population is varied using a variety of approaches, including changing solution concentration for electrospray and changing ion accumulation time. The space-charge effects among Cs^+ are shown to be substantially smaller than those between Cs^+ and other ions, and the frequency-to-intensity relationship can be estimated by a two term linear equation. The discrepancy between these results and Dehmelt's statement about space-charge effects between particles of the same *m*/*z* values is discussed.

2. Experimental

2.1. Material and sample preparation

Cesium iodide (CsI) was purchased from Aldrich (St. Louis, MO), and methanol was purchased from Fisher Chemicals (Fairlawn, NJ). Eighteen MΩ purified water was produced from a Nanopure Infinity system manufactured by Barnstead (Dubuque, IA). Electrospray solutions were prepared using concentrations of CsI from 1×10^{-6} to 1×10^{-3} M in a 70%:30% water:methanol solvent.

2.2. Mass spectrometry

Mass spectra were collected using a 7 T Bruker BioApex-Qh Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer. Ions are generated by electrospray ionization with CsI solutions at flow rates between 10 and 80 μL/h, using the Bruker Apollo II source with a conventional electrospray capillary. Ions are isolated in the quadrupole region and accumulated in the hexapole region for a duration that gives the desired ion intensity. For these experiments, ion accumulation times vary from 0.2 ms to 4.2 s. The ions are then transferred into the FT-ICR analyzer ion cell for detection. For these experiments, no sidekick voltage is applied, and the trapping potential is 1 V. For some experiments, ions are also isolated inside the analyzer ion cell using correlated harmonic excitation field (CHEF) [24]. Ions were detected using a chirp excitation over the mass range of *m*/*z* 20–2000, 100 V_{p-p}, 2375 chirp steps of 20 μs duration per step, 47.5 ms chirp duration. The Cs^+ population in the analyzer cell is controlled by altering the solution concentration, electrospray flow rate and ion accumulation duration in the hexapole.

3. Results and discussion

Since an exact formula relating ion cyclotron frequency and ion population is not known, a direct measurement was made for ions of the same *m*/*z* value. For this study, a population of cesium ions is varied inside the FT-ICR analyzer ion cell by using different methods. Cs^+ is chosen for this study because this element has only one naturally occurring stable isotope, at 132.9 Da, simplifying the production of ions of a single *m*/*z* value. For the data shown in Fig. 1, Cs^+ is generated using electrospray ionization at several CsI concentrations and flow rates. Cs^+ is isolated prior to entering the analyzer cell using an external quadrupole mass filter, and the ions are accumulated in a hexapole following the quadrupole for a fixed duration of 0.2 s. No other ion besides Cs^+ is observed in the mass spectra over the detection range of *m*/*z* 21–2000. To mimic the principal source of ion population fluctuation in a typical experiment, we varied the analyte concentration. As expected, a stronger ion signal is produced by using higher concentrations and/or higher flow rates. The data generated from 1×10^{-3} M (circles) and 1×10^{-5} M (squares) CsI solutions overlap each another and exhibits a linear decrease in the observed cyclotron frequency with an increase in the Cs^+ signal, as shown in Fig. 1. The overall frequency shift is relatively small, 0.4 Hz (0.5 ppm) over the full range of ion intensity variation. The 1×10^{-6} M (triangles) solution produces very weak signals and its data do not overlap with those from the other two concentrations. Furthermore, the frequency-to-intensity behavior does not follow the trend observed for the other two concentrations, and the frequency values are much smaller than expected. This unusual behavior will be discussed later.

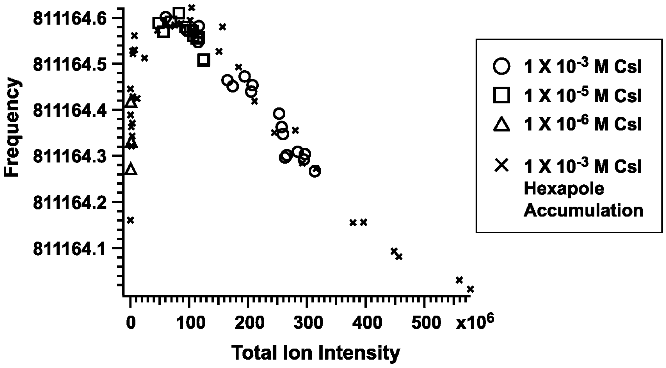

Fig. 1. The measured cyclotron frequency of Cs^+ is plotted as a function of the Cs^+ peak intensity. The ions are generated by using electrospray ionization from solution of 1×10^{-3} M CsI (open circles), 1×10^{-5} M CsI (open squares) and 1×10^{-6} M CsI (open triangles). Cs^+ is selected using an external quadrupole and the ion population is varied by varying the electrospray flow rate. The Cs^+ population is also varied by changing the ion accumulation time in the external hexapole for the 1×10^{-3} M CsI solution (crosses). Linear regression of the data represented by the crosses, with an intensity greater than 100×10^6, gives a slope of -1.35×10^{-9} (0.07×10^{-9} standard deviation) and an intercept of 811164.72 (0.02 standard deviation).

Ion abundance can also be controlled by changing the ion accumulation duration in the external hexapole region and this can be used to investigate space-charge effects in a more controlled manner. Although this method of adjusting the ion population resembles automatic gain control (AGC) [25,26], it has some significant differences from other experimental methods that are used to select ion number. For example, the shape of the ion cloud may be influenced by the hexapole accumulation [17,27,28], and so we have compared these two ways of altering ion population to ensure that the frequency dependence is the same. For this study, Cs^+ is generated using electrospray ionization from a solution of 1×10^{-3} M CsI, and Cs^+ is selected using the quadrupole and accumulated in the hexapole for various time periods, between 0.001 and 2 s, prior to transfer to the analyzer cell. Cs^+ is the only species observed in this experiment. As illustrated in Fig. 1, the resulting cyclotron frequency (crosses) overlaps those acquired using different CsI concentrations and different flow rates, suggesting that ion accumulation in the external hexapole is a reliable method for controlling ion population for space-charge studies. It is important to emphasize that the ion intensity dependence of cyclotron frequency is visibly linear when the intensity is greater than 1×10^8 and that the coefficient of determination (R^2) of a linear regression in this region is determined to be 0.96, though the frequency range spans only 0.6 Hz (0.8 ppm). The frequency variation observed is much smaller than that is usually observed between ions of different *m*/*z* values. The Fisher *F*-test is also used to determine whether the correlation between cyclotron frequency and ion intensity occurred by chance [29]. The null hypothesis is that the correlation coefficient between cyclotron frequency and ion signal is zero, whereas the alternative hypothesis states otherwise. The number of data points with Cs^+ intensity greater than 1×10^8 is 16, thus F_c is determined to be 349. Even at a significance level of 0.1%, the critical $F^*_{(1,14)}$ is 17, much smaller than F_c. Consequently, the correlation between cyclotron frequency and ion intensity is statistically significant at an alpha value of 0.1%. Although, it is not completely certain that the frequency–intensity relationship is purely linear (first-order), a linear term is clearly dominant and that any deviation from the linear relationship is either “noise” or the result of relatively insignificant higher order terms.

Two experiments were performed to confirm that the observed space-charge effects are not an artifact of the quadrupole mass filter used to select Cs^+ prior to ion injection to the analyzer cell. One test was to replace the external quadrupole mass selection with an in-cell correlated harmonic excitation field (CHEF) isolation [24]. The results are shown in Fig. 2. The data from 1×10^{-3} M (circles), 1×10^{-4} M (reversed triangles), 1×10^{-5} M (squares) and 1×10^{-6} M (triangles) CsI solutions show the observed cyclotron frequency increases with increasing ion intensity when the ion abundance is very weak and decreases linearly with increasing ion intensity at higher abundance. The trend is similar to that found for the quadrupole isolation experiment in Fig. 1, although there is a 0.2 Hz constant shift in the observed frequencies as a result of the CHEF isolation. Cs^+ is the only species observed in this experiment. The cyclotron frequency values from the in-cell isolation experiment are higher than those from the quadrupole isolation experiment by ~0.2 Hz and the range of ion intensity is more limited. The CHEF waveform appears to have an effect on the Cs^+ signal, as the Cs^+ intensity decreases after in-cell isolation. This implies that a fraction of Cs^+ is ejected from the analyzer ion cell, which means Cs^+ is kinetically excited by the isolation chirp waveform. Therefore, it is not surprising to observe slightly different cyclotron frequencies from the two isolation experiments, as shown in Figs. 1 and 2. Nonetheless, the in-cell isolation experiment has shown that ion intensity dependence of the cyclotron frequency is not unique to quadrupole ion selection.

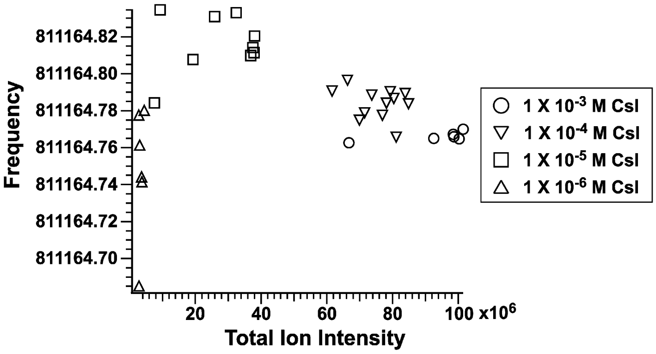

Fig. 2. The measured cyclotron frequency of Cs^+ is plotted as a function of Cs^+ mass peak intensity. Cs^+ is selected by using in-cell isolation and generated from solutions of 1×10^{-3} M CsI (open circles), 1×10^{-4} M CsI (open reversed triangles), 1×10^{-5} M CsI (open squares) and 1×10^{-6} M CsI (open triangles).

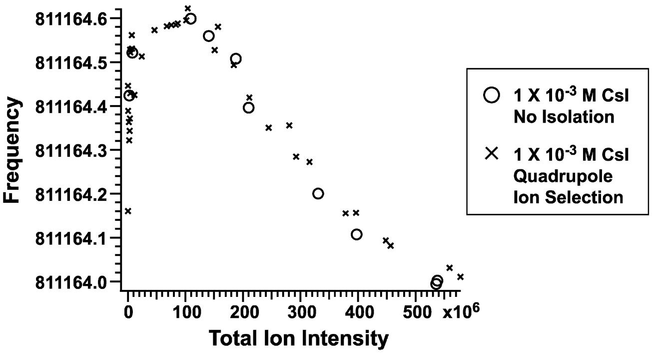

Fig. 3. The measured cyclotron frequency of Cs^+ is plotted as a function of its mass peak intensity. Cs^+ is generated by using electrospray ionization from a 1×10^{-3} M CsI solution and the ions are measured without using quadrupole ion selection (open circles) and using quadrupole selection (crosses). Linear regression of the data represented by the circles gives a slope of -1.44×10^{-9} (1×10^{-10} standard deviation), and an intercept of 811164.74 (0.04 standard deviation.) The data represented by the crosses is the same as the crosses data in Fig. 1.

A second test to eliminate the quadrupole mass filter as contributing an artifact in the frequency shift behavior is to examine the effect of quadrupole selection on the space-charge curve. In Fig. 3, the observed Cs^+ cyclotron frequency generated from a solution of 1×10^{-3} M CsI is plotted against the ion intensity for data acquired in the absence of ion selection (circles) and by using quadrupole selection (crosses). The ion population is controlled by varying the hexapole ion accumulation duration from 0.001 to 3 s. No in-cell ion isolation was used in this experiment. The data for quadrupole selection is the same of that in Fig. 1. The two sets of data overlap each another, which indicates that the quadrupole selection has no measurable effect on the resulting cyclotron frequency. In short, the observed space-charge induced frequency shift is not an artifact of quadrupole selection. The highly concentrated 1×10^{-3} M CsI solution is chosen for this comparative experiment because this minimizes other species in the absence of quadrupole selection: Cs^+ signal constitutes greater than 99% of the total signal. This allows a direct examination of quadrupole selection without concern for space-charge effects from ions of other *m*/*z* value.

In previous space-charge effect studies [15,16,22], mixtures were measured and each species constituted a small fraction of the total ion population, so that the magnitude of space-charge induced frequency shifts between ions of the same *m*/*z* value was difficult to assess with precision. To examine the relative magnitude of space-charge effects between ions of like and dissimilar *m*/*z* value, we have examined the effect for mass spectra dominated by one *m*/*z*, but with other ions present at relatively low abundance. Mass spectra are acquired from a 3×10^{-6} M

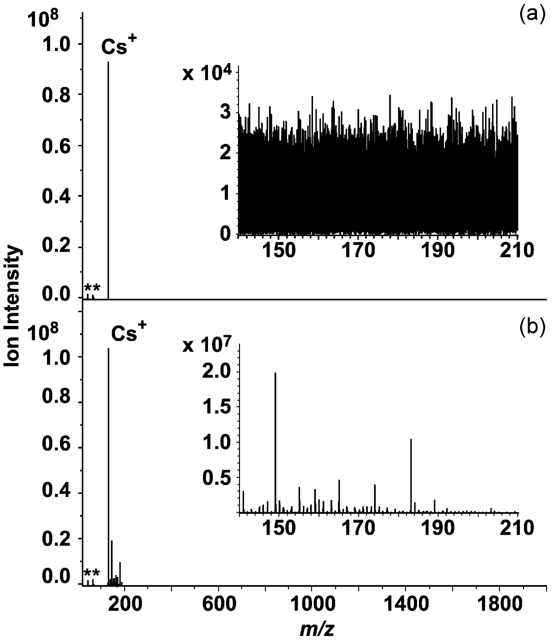

Fig. 4. FT-ICR mass spectra collected from electrospray ionization of 3×10^{-6} M CsI solution by (a) using a quadrupole mass filter for ion selection and (b) without using the quadrupole mass filter for ion selection. The second and third harmonics of Cs^+ signal are denoted with an asterisk symbol.

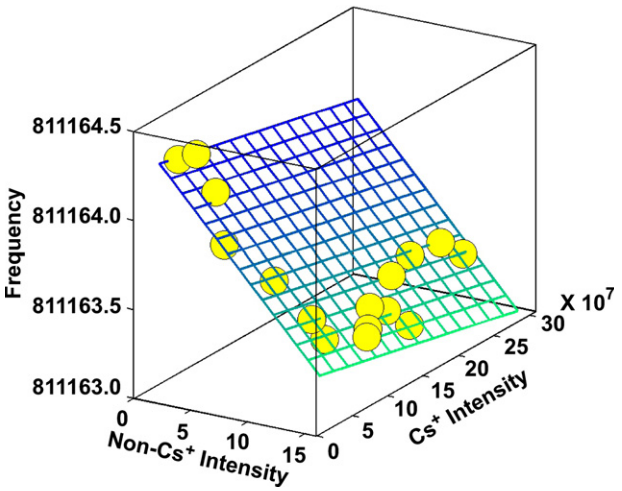

Fig. 6. The measured cyclotron frequency of Cs^+ generated from 3×10^{-6} M CsI solution is plotted against Cs^+ intensity and non-Cs^+ intensity in a three-dimensional plot (spheres). The linear regression plane is illustrated as the grid plane.

CsI solution with and without using the quadrupole mass filter, and ion accumulation is varied between 0.1 and 7.0 s. For spectra collected using mass selection by the quadrupole mass filter, Cs^+ is the dominant species accounting for >98% of the total signal as shown in Fig. 4a. The <2% abundance from other peaks are found at higher *m/z* than Cs^+, and are from ions which are not effectively removed by the isolation process. These additional ions have little impact on the overall space-charge frequency shift, and so the frequency measurements in the isolation experiment from the 3×10^{-6} M CsI solution (open triangles) are found to overlap with those from the 1×10^{-3} M solution (crosses) in Fig. 5. The small signals below the *m/z* of Cs^+ are the second and third harmonics of the *m/z* 132.9 peak along with known electronic noise, and they are not counted as ion signal. For spectra collected in the absence of ion selection, numerous ion species appear between *m/z* 140–1800, as shown in Fig. 4b. Without using quadrupole selection, these species are undetectable at a high concentration of CsI, but appear at higher relative abundance in lower concentration solutions. The cyclotron frequencies observed without using quadrupole selection (filled triangles) have a discernibly stronger dependence on the total ion signal compared to those obtained using quadrupole selection (open triangles), as shown in Fig. 5. The cyclotron frequency obtained without using ion selection (filled triangles) has a complex dependence on the total ion intensity. There is a linear region with a negative slope at low total ion intensity, and a region of near zero slope at higher intensity. This is because the ion intensity ratio between Cs^+ and non-Cs^+ species is variable. The intensity of the non-Cs^+ species (line) increases with total ion intensity when the total ion intensity is below 2×10^8, and decreases beyond that (Fig. 5). The cyclotron frequency is better described as linearly dependent on intensities for ions of the same *m/z* value and ions of different *m/z* value, but with a larger dependence for ions of different *m/z* value. Consequentially, the ion intensity dependence of cyclotron frequency will appear to flatten out at high total intensities when the ion accumulation times are longer and when the non-Cs^+ abundance actually decreases. These effects are more clearly observed in Fig. 6, where the observed cyclotron frequency (spheres) is plotted against ion intensities of Cs^+ and non-Cs^+ in a three-dimensional graph. Data is fitted with linear regression using a two variable equation

$$f = AI_{\mathrm{Cs}} + BI_{\mathrm{others}} + C \tag{4}$$

where *f* is the measured cyclotron frequency of Cs^+, I_{Cs} the ion intensity of Cs^+, I_{others} the accumulated ion intensity of non-Cs^+ species, and *A*, *B*, *C* are the fitting parameters. The resulting regression of Eq. (4) is represented as the grid plane in Fig. 6, which is a good fit to the experimental data. The space-charge

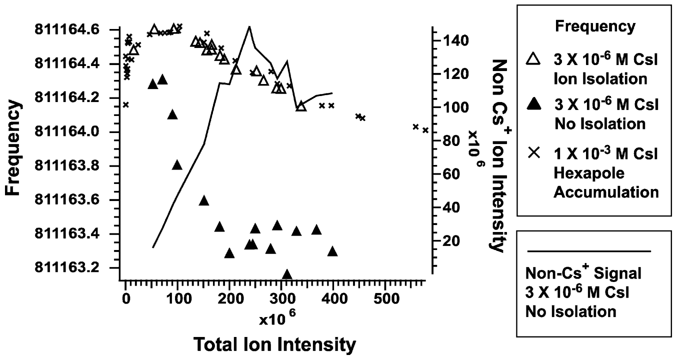

Fig. 5. The measured cyclotron frequency is plotted against total ion intensity for mass spectra collected from 3×10^{-6} M CsI solution using quadrupole selection (open triangles) and without using quadrupole selection (filled triangles) and from 1×10^{-3} M CsI solution using quadrupole selection (crosses). The non-Cs^+ signal intensity from mass spectra acquired from 3×10^{-6} M CsI solution without ion selection is plotted against total ion intensity (line).

dependence on the observed frequency of Cs^+ is about 7.5 times stronger from the non-Cs^+ species than from Cs^+, as estimated through the regression values of A and B parameters in Eq. (4), which appear as the slopes in Fig. 6, -9.61×10^{-10} (for the frequency dependence on the Cs ion) and -7.17×10^{-9} (for frequency dependence on non-Cs ions). Both lines have an intercept of 811164.38.

From these results, the space-charge frequency shift for Cs^+ is demonstrated to be linearly proportional to the ion intensity of Cs^+ itself, and linearly proportional to the ion intensity of other ions at a greater magnitude. Consequently, if space-charge frequency shift is solely estimated based on the total ion intensity for a spectrum with a distribution of peak intensities, then the frequency shift of each peak will deviate linearly by the difference between its intensity and the average peak intensity. The frequency shift of the high intensity peaks will be overestimated, producing negative mass errors, while the frequency shift of the low intensity peaks will be underestimated, producing positive mass errors, a phenomena noticed by Smith and coworkers [15]. This also explains the reason why the calibration equations (1)–(3) developed by Eyler, Smith, Muddiman and our groups [14–16,22] are able to reduce mass error by incorporating a local space-charge term that is linearly proportional to the ion intensity of each mass. In reality, the space-charge frequency shift probably includes higher order terms, such as I_i^2 and $I_i I_{\text{others}}$, but these terms appear to have much smaller effect and will be much more difficult to extract. The work demonstrated in this paper gives some physical meaning to the local space-charge calibration equations.

It is important to recognize that the intensity dependence of Cs^+ cyclotron frequency is not the result of incomplete ion isolation, although it is difficult to confirm the complete removal of all non-Cs^+ ions (because low abundance ions may exist without being detected in the mass spectra). In Fig. 3, the cyclotron frequency data from experiments with and without quadrupole selection are essentially the same. If undetected ions were responsible for the ion intensity dependence of the frequency, then the two sets of data would exhibit differences, as the mass filter should still be able to remove the majority of non-Cs^+ species. Thus, non-Cs^+ species are not responsible for the space-charge relationship observed in quadrupole selection experiments.

We do not have a concrete explanation for the observed increase in cyclotron frequency at very low ion intensity in Figs. 1–3. An important assumption we make here is that the mass spectral signal is proportional to the ion population in the analyzer cell, but this may not be accurate at very low ion populations. Nikolaev et al. have made ion trajectory calculations that suggest that ion packets dephase at a higher rate at low ion population [30]. Thus, we may have a systematic error in the estimation of ion number at low ion intensities. The odd behavior of observed frequency at very low ion populations merits further study.

Our data clearly contradict the notion that ions of the same m/z value cannot frequency shift each other. There are many reasons that the statement made by Wineland and Dehmelt [19] is not applicable to most FT-ICR experiments. Most importantly, Wineland and Dehmelt focused their theory and experiment on the trapping oscillations, i.e., motion parallel to the magnetic field. The statement about the motion perpendicular to the magnetic field was made as an after-thought in a second paper [19]. The center-of-mass argument is justified only when the forces between particles completely cancel both in magnitude and direction (i.e., cancellation of equal and opposite forces.) Axial motion is constant with respect to the number of electrons because a pure electrostatic force provides a central force, in which the opposing forces act through each other directly, allowing the electric forces between charged particles to cancel. However, motion perpendicular to the magnetic field is entirely different, as the Lorentz force depends on the velocities of the interacting charged particles and is noncentral, or in other words, the force vector does not align with an axis that connects the two particles [31–34]. Although the total momentum is conserved, the mechanical momentum between the charged particles does not have to be conserved because the electromagnetic field can carry momentum. The results can be rationalized by recognizing that the center-of-mass motion in FT-ICR mass spectrometry conforms to the weak form of the Newton's third law, where the action and reaction forces do not exactly cancel. Consequentially, the forces between ions of the same m/z do not cancel completely, in contradiction to the theory forwarded by Wineland and Dehmelt, and space-charge frequency shifts can occur. Cornille has developed a theory that shows that Lorentz forces do not obey the strong form of Newton's third law (cancellation of opposing equal forces), by illustrating with a pair of charged particles in relative motion which can change its state of motion [35]. In addition, the ion cloud is expected to expand as the number of ions increases, which allows the ions to sample more of the cell parallel to the magnetic field, and to be exposed to a wider distribution of radial electrical fields. The amount of induced charge on the excitation and detection plates is also expected to increase with the number of ions, causing a greater radial outward electric field. The cyclotron frequency is expected to decrease in either case. Similar points have been made by Jeffries et al. [10].

4. Conclusions

A better understanding of space-charge induced frequency shifts in FT-ICR mass spectrometry can improve mass calibration equations and consequently increases mass measurement accuracy. Since the induced frequency shift is smaller between ions of the same m/z value than between ions of different m/z value, new mass calibration equations have been formulated for the overcorrection of the space-charge effects between ions of the same m/z value by including the "local space-charge" term [14–16,22]. The two space-charge effects are directly demonstrated through the frequency shift of Cs^+ by its own population and by non-Cs^+ population. Although the space-charge effects between ions of the same m/z value are small, the relationship is statistically significant and cannot be neglected. Despite the previous prediction about the improbable existence of cyclotron frequency shift between ions of the same m/z value [19], such frequency shifts are reasonable because the mechanical momentum of ions can interact with the momentum of the surrounding electromagnetic fields.

Acknowledgments

The authors would like to acknowledge the many contributions of Professor Jean Futrell to the fields of ion chemistry, ion physics and mass spectrometry. The authors would also like to thanks Professor Lutz Schweikhard, Dr. Christophe Masselon and Dr. Alexander Friedland for helpful discussions. The authors are also grateful for the financial support through National Science Foundation (grant CHE-0316002) and National Institutes of Health (grant R01-RR 0119767).

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