High-Resolution Ion Isolation with the Ion Cyclotron Resonance Capacitively Coupled Open Cell

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For ion cyclotron resonance, a capacitively coupled open cell variant with fourfold radial symmetry was constructed and tested for axial excitation–ejection of large ions at high resolution. With a reverse of frequency sweep direction, this cell gave substantial improvements in signal-to-noise ratio due to linearization of the excitation electric field. Single isotopic peaks of ubiquitin (8.6-kDa) and carbonic anhydrase (29-kDa) molecular ions could be isolated by selective stored waveform inverse Fourier transform excitation, which yielded an order of magnitude higher isolation resolving power than previously achieved at high mass-to-charge ratio values. *(J Am Soc Mass Spectrom 1995, 6, 533–535)*

One significant limitation in Fourier transform mass spectrometry (FTMS) is inhomogeneous excitation electric fields, which are responsible for spectral problems such as line broadening, amplitude variance, and sidebands that can arise from coupling of axial and cyclotron modes during rf excitation [1–3]. Such problems are particularly critical for electrospray ionization–FTMS mass spectra of large biomolecules, because isotopic resolution [4, 5] and accurate isotopic distribution amplitudes [6a] are often required for interpretation of these complex ion mixtures.

The frequency sweep direction of the excitation electric field has been demonstrated to determine the extent of axial excitation: irradiation at $2\omega_z$ and $\omega_+ + 2\omega_z$ excites the axial translational energy mode [7, 8]. [ω_z is the trapping oscillation frequency and ω_+ is the perturbed cyclotron frequency as described by the equation $\omega_\pm = nqB/2m \pm (n^2q^2B/4m^2 - nqE/m)^{1/2}$ [6b], where n is the charge state, E is the electric field, B is the magnetic field, and m and q are the mass and fundamental charge, respectively.] Displacement from the cell center shifts the cyclotron frequency [9], which rapidly reduces the ion bundle coherence, ion signal, and resolution. Also, stored waveform inverse Fourier transform (SWIFT) ejection [10] of ions outside of a specific mass-to-charge ratio range is fundamentally limited by axial modulation of the ions' resonant frequencies during the SWIFT isolation event. One method for minimization of the axial component to the excitation field is capacitive coupling of the open trapped ion cell [3]; this is implemented here for large biomolecule spectra. All spectra were taken on a 6.1-T external injection electrospray ionization–FTMS previously described [12]. Trapping was accomplished with 4 and 5 V on the source and analyzer trap plates, respectively, during a N_2 pulse peaking at 1×10^{-6} torr, after which the values were dropped to 1 V. Excitation for detection used a 50–150-kHz, 240-Hz/μs linear frequency sweep or "chirp" that corresponded roughly to m/z 600–1800 and 150-kHz bandwidth detection.

Capacitive coupling extends the excitation field onto the trap plates of the cell, to more closely approximate an infinitely extended rf excitation electric field. Coupling to the trap plates is performed by placement of capacitors and resistors on the cell (Figure 1), which provides a high pass filter for the rf excitation fields and a low pass filter for the trapping fields. The 3 dB rolloff point for this filter was set at 1600 Hz [10-nf capacitors (muRata Electronics part number GRM40-X7R103K050BD); 10-kΩ inductiveless carbon resistors; rise time = 0.25 ms]. Previous cells used capacitive coupling on the excitation plates exclusively [3], but here the detection electrodes also were coupled to preserve fourfold radial symmetry for quadrupolar axialization [12–14]. Such coupling also should increase the detection efficiency slightly due to the larger effective surface area of the detection plates [2, 15]. However, the 10-kΩ trapping impedance, which is much lower than the 10^6–10^8 Ω input impedance to the preamp, is likely to drop the detection power collected on the segmented trapping electrodes through the trapping power supplies instead of through the

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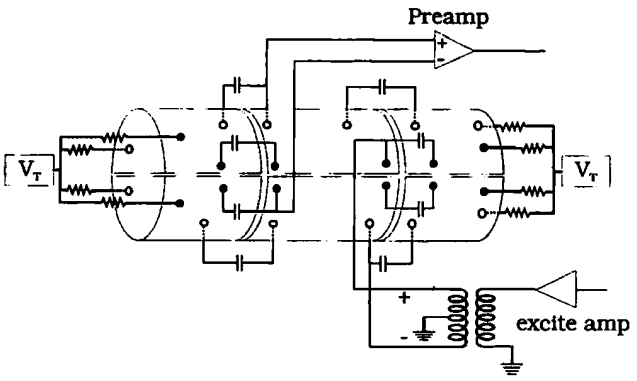

Figure 1. Circuit diagram of the capacitively coupled cylindrical open trapped-ion cell. Cell diameter is 5.65 cm and each of the 12 individual cell segments are 5.84 cm long.

preamp. Further experiments that use 10-MΩ resistors are planned. It also was necessary to place a relay to ground on the detection lines so that the detect plates could be discharged after rapid changes in trapping voltages. The output impedance for the excitation circuit is inductive, whereas the input impedance for the preamplifier is resistive and effectively infinite, which results in dramatically different time constants for the respective filters. Without the relay, rapid changes to trapping potentials greatly distort the cell static electric trapping fields, which results in ion loss.

To test excitation linearity, frequency sweep excitations in both directions were applied to both cells. Without capacitive coupling, sweeping from high to low frequency causes significant attenuation (poorer signal-to-noise ratio) in the ion signal and the resultant spectrum (Figure 2b) relative to the reverse sweep direction. Although it is contrary to both excitation theory and experiment [7, 8, 16], this effect must be due to greater axial ejection, because the signal-to-noise ratio (while sweeping high to low) is improved by increased trapping potentials or reduced excitation amplitude. With capacitive coupling, a change in the sweep direction does not significantly alter the signal-

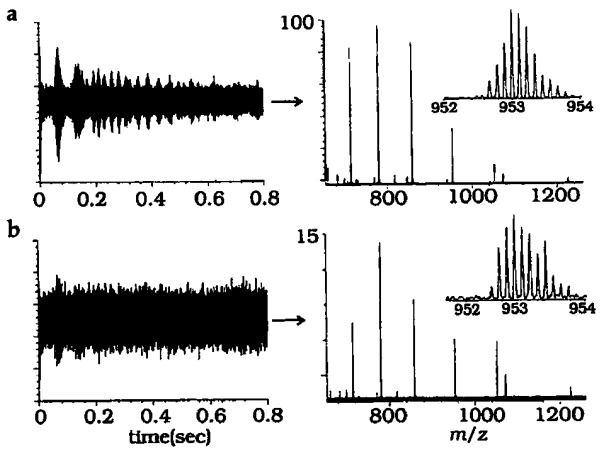

Figure 2. The effect of excitation sweep direction for the cylindrical open cell: (a) low to high frequency; (b) high to low frequency.

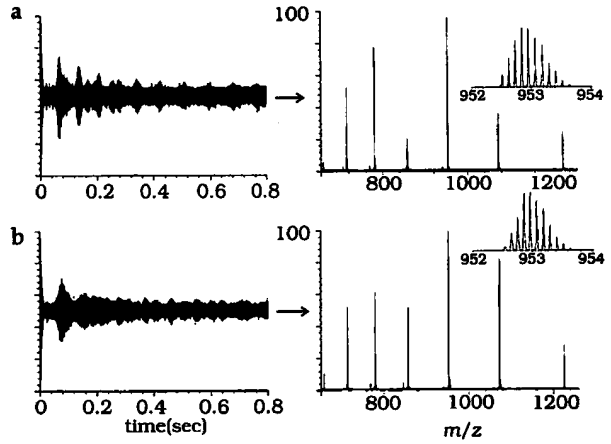

Figure 3. The effect of sweep direction for the capacitively coupled cylindrical open cell as in Figure 2.

to-noise ratio (Figure 3). The remaining differences in intensity between the two excitations (Figure 3a versus Figure 3b) could arise from several sources, which include nonquadrupolar trapping potentials, differences in instantaneous space–charge effects as ions are excited in different orders, and imperfect waveform generation. Several experiments are planned to determine the true nature of these differences.

Excitation field inhomogeneity that causes axial mode coupling also reduces resolving power with SWIFT dipolar excitation for isolation of precursor ions in tandem mass spectrometry experiments. However, with the capacitively coupled open cell, isolation of a single isotope of ubiquitin (8.6 kDa; Figure 4) and carbonic anhydrase (29 kDa; Figure 5) was accomplished on two separate isotopic peaks by using either a single 4-MB SWIFT waveform with calculated resolving power (RP) of 5×10^4 or two waveform—one to isolate an individual charge state (128 kB, 175-kHz bandwidth) and the other a high resolution heterodyne waveform (2048 kB, 62.5 kHz). The individual isotopic peaks require RP of 8600 and 29,000 to separate them

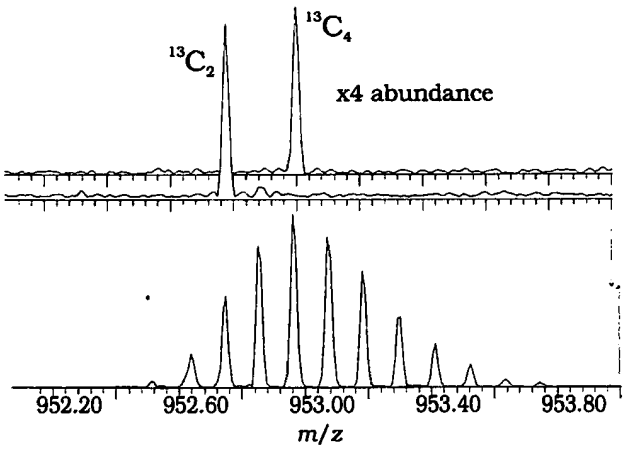

Figure 4. SWIFT isolation of ubiquitin (8.6-kDa) isotopic peaks that contain two and four ^{13}C atoms. The frequency shift denoted by the arrows on the right is due to the magnetic field drift of 4.8 ppm/h.

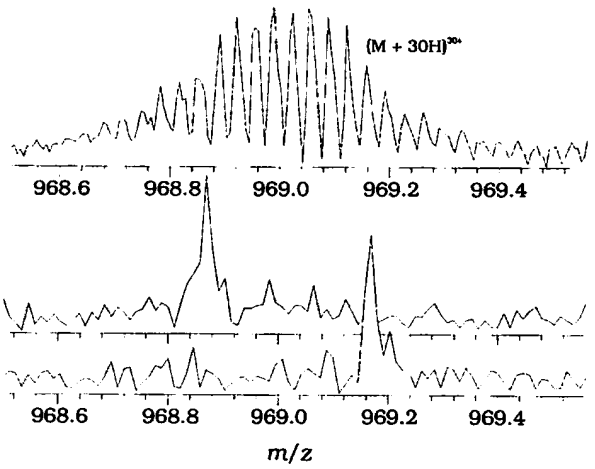

Figure 5. SWIFT isolation of two separate single isotope peaks of carbonic anhydrase (29 kDa).

from their adjacent isotope peaks; all but ~ 2 and ~ 30%, respectively, of the adjacent peaks are removed. The previously reported maximum isolation RP at high mass-to-charge ratio required quadrupolar excitation to isolate a single isotope from C_{84} [13] (RP = 1000) and at low mass-to-charge ratio, isolation of mass 92.062 from 92.058 (RP = 21000) was accomplished on a 3-T system with all but ~ 1% of adjacent peaks removed [17].

Coupling the open cell has, therefore, greatly increased excitation field homogeneity to allow improved detection and isolation of single precursor peaks for tandem mass spectrometry dissociation. The latter could provide valuable information for identification of the isotopic composition of such a selected peak [18]. For example, an $[M + nH]^{n+}$ isotopic peak of apomyoglobin can be measured with a few millidaltons accuracy [19], but misassignment of its isotopic composition would yield a 1-Da error. However, isolation of the $^{13}C_2$ ions of ubiquitin followed by cleavage of these ions in half would give $^{13}C_0$:$^{13}C_1$:$^{13}C_2$ abundances of 1:2:1 ratio, whereas cleveage of the $^{13}C_4$ ions would yield fragment ions in a 1:4:6:4:1 ratio [18]. Future research directions with the capacitively coupled cell include quadrupolar excitation for remeasurement and improved tandem mass spectrometry efficiency.

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