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High-Performance Mass Spectrometry: Fourier Transform Ion Cyclotron Resonance at 14.5 Tesla

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We describe the design and current performance of a 14.5 T hybrid linear quadrupole ion trap Fourier transform ion cyclotron resonance mass spectrometer. Ion masses are routinely determined at 4-fold better mass accuracy and 2-fold higher resolving power than similar 7 T systems at the same scan rate. The combination of high magnetic field and strict control of the number of trapped ions results in external calibration broadband mass accuracy typically less than 300 ppb rms, and a resolving power of 200 000 ($m/\Delta m_{50\%}$ at m/z 400) is achieved at greater than 1 mass spectrum per second. Novel ion storage optics and methodology increase the maximum number of ions that can be delivered to the FTICR cell, thereby improving dynamic range for tandem mass spectrometry and complex mixture applications.

The highest mass resolving power and mass measurement accuracy in analytical mass spectrometry is provided by Fourier transform ion cyclotron resonance (FTICR).[1] Resolving power greater than $m/\Delta m_{50\%} = 100\,000$ (in which $\Delta m_{50\%}$ is the full peak width at half-maximum peak height) and mass accuracy better than 1 part per million (ppm) have facilitated new methodology in a variety of fertile bioanalytical fields and initiated completely new analytical fields. Examples of recent biological applications that exploit the unique performance of FTICR are lipidomic analysis,[2] de novo peptide sequencing,[3–5] hydrogen/deuterium exchange (HDX),[6–9] bottom-up proteomics,[10] and characterization of post-translational modification of peptides and proteins.[11–13] High mass accuracy improves identification confidence and limits search space for faster data processing in proteomic analyses.[14,15] For HDX experiments, the overlap of peptide isotopic distributions (even for LC-separated peptides) increases on deuterium uptake, and high resolving power is required to properly track such signals. In fact, high-field FTICR enables direct analysis of H/D exchange data based on elemental composition assignment, an approach not available to lower resolution instruments.[16] Furthermore, high-field FTICR enables high resolving power and high scan rate *simultaneously*, to minimize back-exchange and overlap of peptide isotopic distributions. The emerging field of "top-down" proteomics[17–19] relies heavily on high-performance mass analysis, and recently developed entire new protocols are based on high-field FTICR. For example, it is now possible to record high resolution, high mass accuracy MS and MS/MS data online for intact proteins separated by LC.[20,21] Direct infusion of complex biological samples has been recently shown for a 7 T linear quadrupole ion trap (LTQ) FTICR system by use of sequential

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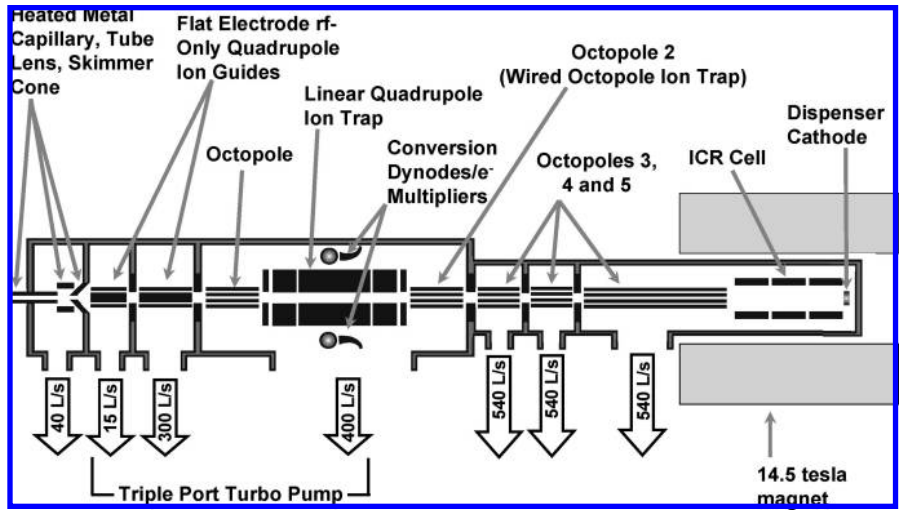

Figure 1. Schematic diagram (not to scale) of the 14.5 T hybrid linear quadrupole ion trap/FTICR mass spectrometer. The wired octopole enables a second mode of mass-selective ion accumulation whereby ions are mass selected in the LTQ and transferred to the adjacent storage octopole. That process can be repeated for numerous cycles before the entire octopole ion population is transferred to the ICR cell.

quadrupole ion trap isolation of narrow m/z segments to improve the dynamic range.[22] Such analyses benefit from the high peak capacity of FTICR MS, (especially if many time-domain signals may be collected and summed), and at high magnetic field strength quadrupole isolation may not be necessary.

High-field FTICR also fosters exciting advances in the analysis of petroleum[23,24] and natural organic matter.[25,26] Because of sample complexity, those analyses benefit especially from the dynamic range and resolving power improvements realized at high magnetic field.[27] Even at the ultrahigh resolving power ($m/\Delta m_{50\%}$ = 400 000 at m/z 500) achievable at 9.4 T, unresolved multiplets increase in frequency at high m/z, so higher resolution is necessary. Moreover, enhanced mass accuracy raises the upper mass limit for unambiguous elemental composition assignment.[23,28]

Here, we describe the design and capabilities of a 14.5 T hybrid linear quadrupole ion trap/FTICR mass spectrometer. High magnetic field results in higher resolving power, acquisition rate, mass accuracy, and dynamic range relative to similar instruments at lower field. A key feature of the instrument is automatic gain control (AGC)[29] to precisely select the number of ions measured in each FTICR scan, thereby minimizing the need to internally calibrate mass spectra. AGC coupled with high-magnetic field FTICR is particularly powerful for reducing scan-to-scan variation in the number of trapped ions, and high magnetic field minimizes the observed shift in ion cyclotron frequency that results from residual variation in the number of trapped ions. Finally, because cyclotron frequency increases linearly with magnetic field strength and the magnitude of space charge induced frequency shifts decreases with increased magnetic field strength, we demonstrate the higher order (quadratic) dependence of externally calibrated mass accuracy on magnetic field strength.

EXPERIMENTAL METHODS

Instrumentation. Our instrument (see Figure 1) is a hybrid linear quadrupole ion trap/FTICR MS (LTQ-FT, Thermo Fisher Corp., Bremen, Germany) adapted to operate in an actively shielded 14.5 T superconducting magnet (Magnex, Oxford, U.K.). The magnet bore diameter is 104 mm with an axial distance of 880 mm from the nearest flange to field center and 1966 mm overall length (i.e., the magnetic field center is offset axially). The magnet employs a set of eight superconducting shims to minimize spatial magnetic field gradients through first order and an additional set of ferroshims for higher order gradients to achieve an overall spatial inhomogeneity of 2.6 ppm peak variation on the surface of a 60 mm diameter by 60 mm long cylinder. The fringe magnetic field is approximately 75 gauss at a distance of 1.5 m from field center in any direction, substantially higher than most (shielded) FTICR magnets.

Ultrahigh vacuum is more critical at high magnetic field because high ion kinetic energy (which scales as the square of the magnetic field)[27] can cause unintended collisional dissociation during detection, especially for ions of low m/z (kinetic energy and collision rate (hard sphere model[30]) scale inversely with m/z) and/or high postexcitation radius (kinetic energy scales with the square of the ICR orbital radius). Early measurements with our system suggested m/z dependent collisional dissociation of ions during ICR detection. Therefore, we added an extra stage of differential pumping (with an additional octopole ion guide) to reduce the helium gas load delivered from the LTQ to the ICR cell. Further, the three lowest pressure stages are equipped with high speed, high compression (580 L/s; 10^8 compression ratio for helium) turbomolecular pumps (Edwards EXT 556HF, Wilmington, MA); the pump bodies are made from martensitic

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steel to shield the rotating blades from the magnet fringe field). The combination decreases the measured base pressure 10-fold (2×10^{-10} Torr, as measured by a Bayard−Alpert ionization gauge) and reduces unwanted collisional dissociation, particularly for low m/z ions.

Although the ion optics between the linear trap and the ICR cell are modified, the instrument maintains the full functionality of the standard commercial system (automatic gain control, data dependent MS and MS^n, parallel ICR/linear trap detection, etc.). The octopole directly behind the LTQ has been modified to incorporate tilted wire extraction electrodes,[31] for improved ion ejection efficiency and introduction of a second mode of external ion accumulation (see Results and Discussion). The final octopole of our system is lengthened to accommodate the longer distance to field center at 14.5 T relative to 7 T. We have optimized ion transmission between the linear trap and ICR cell with in-house written scripts. Voltages (rf and dc offset) for octopoles 1, 3, and 4 (Figure 1) are supplied by the tuned circuit oscillators of the standard system, whereas octopole 2 is driven by a high power rf supply (T&C Power Conversion, Rochester, NY) coupled through a center-tapped transformer (Northhills, Syosett, NY). The ICR cell is open cylindrical[32] with a diameter of 55 mm and an axial trapping dimension of 82 mm (i.e., aspect ratio ~1.5). The cell was supplied by the vendor and has been rewired for external capacitive coupling.[33] Software specified waveforms for dipolar excitation are input from the instrument control PCB to a high power rf supply (ENI, Rochester, NY) coupled through a center tapped transformer (Northhills, Syosett, NY) and input to a custom-built circuit for distribution of voltages that facilitate capacitive coupling. Instrument parameters are controlled by the commercial software (Xcalibur, Thermo Fisher, San Jose, CA). Available fragmentation methods include collisionally activated (CAD) in the linear trap and electron capture (ECD)[34] in the ICR cell. Microelectrospray[35] is performed with standard hardware, and atmospheric pressure photoionization (APPI)[36] is performed with a commercial ion source (IonMax, Thermo Fisher, San Jose, CA).

Data Analysis. Mass spectra are analyzed with commercial software (Xcalibur, Thermo Fisher, San Jose, CA) for experiments that do not require signal averaging (e.g., LC). For petroleum analysis and other measurements for which signal averaging is required, ICR time-domain transients are recorded individually, then added, Hanning apodized, zero-filled once, and fast Fourier transformed with MIDAS analysis software.[37]

Samples and Chromatography. Standard compounds (myoglobin from equine heart, protease type XIII from *Aspergillus saitoi*, substance P, β-casomorphin, Ultramark, MRFA peptide, and caffeine) were purchased from Sigma Aldrich. A Middle East crude oil was obtained from ExxonMobil and dissolved to 0.5 mg/mL in toluene for atmospheric pressure photoionization. To demonstrate broadband mass measurement accuracy for LC-separated compounds, 4 μM myoglobin in 50 mM sodium phosphate buffer (pH = 8.0) was enzymatically digested at an initial protein/protease type XIII ratio of 10. After digestion, the protein digest was injected from a 10 μL loop onto a 1 mm × 50 mm C column (Phenomenex) and a gradient (2% B to 95% B in 6 min; A, acetonitrile/H_2O/formic acid 5/94.5/0.5; B, acetonitrile/H_2O/formic acid 95/4.5/0.5) was used to elute the peptides. The eluent was postcolumn split and infused by microelectrospray ionization into the mass spectrometer.

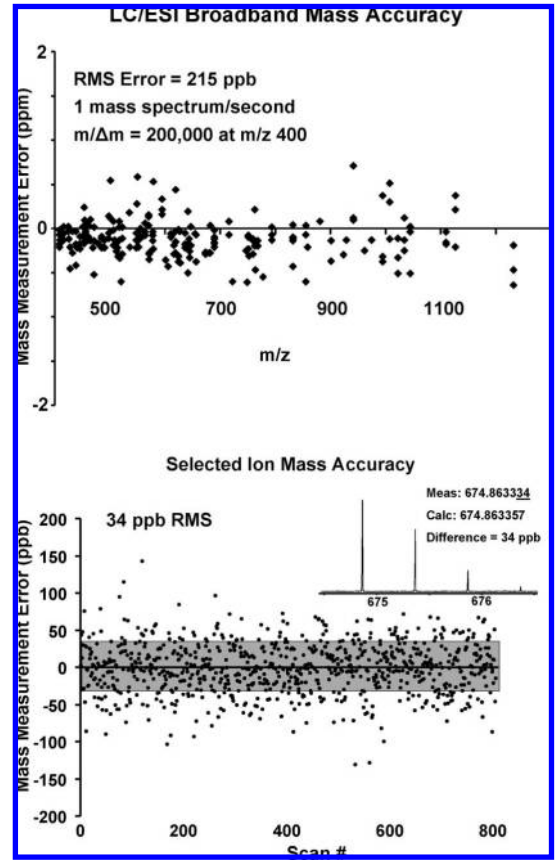

Figure 2. (Top) Typical broadband external calibration mass accuracy for 14.5 T LC−ESI FTICR MS demonstrated for known peptides of digested equine myoglobin (three experiments). FTICR mass spectra were collected with 1 million ions/measurement at high mass resolving power and acquisition speed during an 8 min LC program. (Bottom) Typical narrowband (selected ion monitoring, SIM) external calibration mass accuracy demonstrated for 800 measurements of substance P at 500 fmol/μL (target ion population of 500 000 ions/measurement and a quadrupole ion trap selection window of $\Delta(m/z) = 10$).

RESULTS AND DISCUSSION

Mass Accuracy. Figure 2 (top) shows typical broadband external calibration mass accuracy for online LC−MS, illustrated here for an enzymatic digest of horse heart myoglobin (triplicate analysis). Until now, the relationship of mass accuracy to magnetic field strength was difficult to determine, primarily due to differences in instrument design and experimental methods for FTICR systems of different field strength. However, comparison of the present 14.5 to 7 T LTQ FTICR instruments shows that mass accuracy scales as the square of magnetic field strength. For example, the commercial specification

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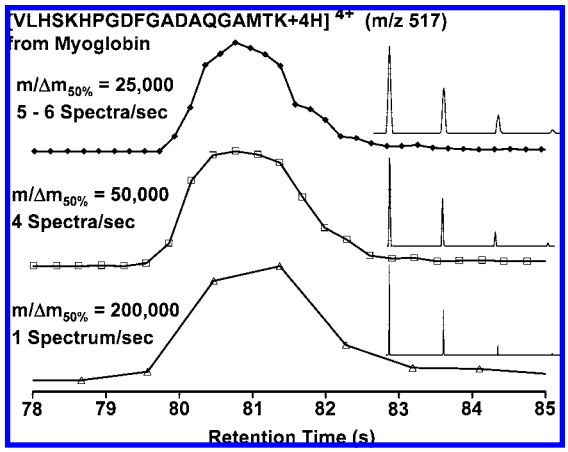

Figure 3. Selected ion chromatograms of the peptide, [VLHSKHPGDFGADAQGAMTK + 4H]$^{4+}$, for FTICR spectral acquisition frequency: 6 (top), 4 (middle), and 1 (bottom) spectra/s. FTICR mass spectra are shown in the zoom insets.

for mass accuracy for the commercial 7 T LTQ-FT is 2 ppm rms (m/z 400, 1 s duty cycle) for broadband analyses, and 1−2 ppm has been reported.[29] Typical routine results from our 14.5 T system are 200−300 ppb (Figure 2, top).

Similarly, we observe unprecedented selected-ion mass accuracy (34 ppb rms error) as illustrated in Figure 2 (bottom) for 800 measurements of substance P (500 fmol/μL, not present in the calibration solution) selected from a mixture of five standard peptides. For selected ions, our results are roughly 2-fold better than at 7 T (not 4-fold), because selection of a single m/z species reduces the space charge shift due to reduction in ion number by 2 orders of magnitude.[38] Moreover, we note that rms error below ~50 ppb requires determination of peak position as a double precision number (single precision data reduction results in m/z values that are quantized in increments of 0.000 06). The present data was collected at ~1 spectrum/s, and that level of performance is thus available with direct infusion as well as data-dependent LC operation.

Data Acquisition Speed. The rate at which FTICR data can be acquired increases linearly with magnetic field strength. At 14.5 T, a time-domain acquisition period of 767 ms provides mass resolving power of $m/\Delta m_{50\%}$ = 213 000 (at m/z 400). A typical period of ~100 ms (or less) for external ion accumulation results in spectra with high mass resolving power collected at a rate greater than 1 spectrum per second (i.e., the benchmark for chromatographic sampling) as shown by a selected-ion mass chromatogram in the lower portion of Figure 3. The selected ion chromatograms displayed are for the quadruply charged myoglobin fragment peptide, [VLHSKHPGDFGADAQGAMTK + 4H]$^{4+}$, as observed from a steep-gradient LC−FTICR MS measurement. Alternatively, high magnetic field and efficient external ion accumulation allow faster chromatographic sampling at reduced (but still impressive) resolving power. For example, the upper two selected ion chromatograms of Figure 3 illustrate mass resolving powers of 50 000 and 25 000 (m/z 400) for chromatographic sampling at 4 and ~6 mass spectra/s. Note that the acquisition period required to achieve $m/\Delta m_{50\%}$ = 25 000 at m/z 400 is 90 ms, which allows for up to ~11 acquisitions/s. Although the maximum acquisition frequency for experiments such as in Figure 3 is limited by the ion accumulation period, the full theoretical limit may be achieved by simultaneous ion accumulation and detection.[39]

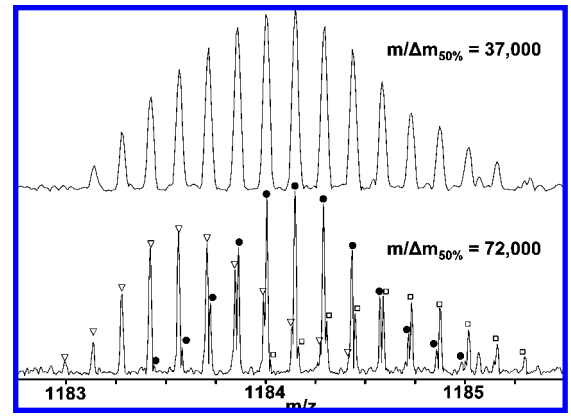

Figure 4. Mass scale expanded segments from a positive-ion electrospray FTICR mass spectrum of insect neuropeptides collected at 1 scan/s and 200 000 resolving power (m/z 400). Bottom: resolved overlapping isotopic distributions for three 8 kDa proteins. Top: The same m/z segment from FT of the same time-domain data truncated by half (i.e., equivalent to halving the resolving power), for which the three isotopic distributions are no longer resolved.

Mass Resolving Power. Figure 4 illustrates the importance of a factor of 2 improvement in mass resolving power in biological analyses. The lower panel shows an m/z scale-expanded segment from a direct infusion positive-ion electrospray FTICR mass spectrum collected at 1 spectrum/s ($m/\Delta m_{50\%}$ = 207 000 at m/z 400 and 70 000 at m/z 1183) for a complex mixture of neuropeptides and intact proteins extracted from *Periplaneta americana* (cockroach) brain.[40] The upper trace was generated by truncating the time domain by half to simulate what would be observed if this sample were analyzed at the same acquisition rate but with a 7 T FTICR mass spectrometer. The three overlapping isotopic distributions each originate from 7+ ions of similar mass (8281.012, 8284.121, and 8288.189 Da, measured) that likely derive from amino acid substitution and/or post-translational modification of similar structures. Such compounds are not always separated by chromatography, and in the absence of high-resolution mass analysis, the identity and number of species present would be incorrectly determined. In fact, we commonly observe overlapping isotopic distributions of peptides and proteins in LC−MS analyses, and examples of such instances have been published (also collected with the NHMFL 14.5 T LTQ-FTICR mass spectrometer).[6,16] The three peptides in this example are clearly resolved at 14.5 T, but would not be resolved at 7 T. Thus, a factor of 2 in resolving power is not just twice as good, it can be the difference between obtaining an answer or not.

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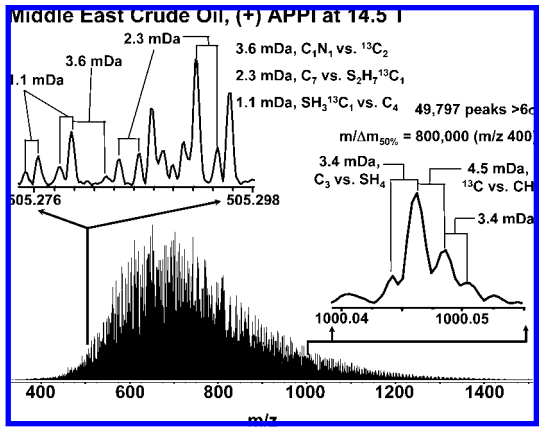

Figure 5. Positive-ion atmospheric pressure photoionization FTICR mass spectrum of an Arabian light crude oil. Nearly 50 000 peaks are detected across the range 340 > *m*/*z* < 1500. Zoom insets illustrate resolution of numerous mass doublets separated by as little as 1.1 mDa (top) and resolution of the C_3 vs SH_4 mass doublet (3.4 mDa) up to *m*/*z* 1000 (bottom).

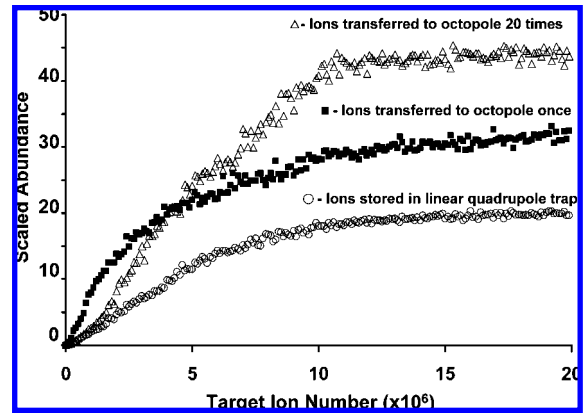

Figure 6. Abundance versus accumulated ion number (*m*/*z* 864) for three selected ion accumulation schemes. Initial results show a 2−3-fold improvement in ion storage capacity for selected ions ejected from the wired octopole rather than those selected, stored, and transferred directly from the LTQ.

Complex Samples: Combined Dynamic Range and Resolution. A major advantage of the 14.5 T instrument over lower field instruments is reduced sensitivity to space charge effects. The ability to tolerate large ion populations is especially critical for petroleum analysis, for which the compositional complexity of the sample dictates that the spectrometer achieve ultrahigh resolving power and mass accuracy over a wide dynamic range. Figure 5 illustrates that capability for positive-ion atmospheric pressure photoionization of a Middle Eastern light crude oil. The mass spectrum is generated by offline summation of 500 individual 3 s time-domain transients. The achieved resolving power of 800 000 at *m*/*z* 400 results in 49 797 detected peaks, each with a magnitude greater than 6σ of baseline noise, and all but ~600 could be grouped into homologous series. The large number of peaks at high mass makes elemental composition assignment more difficult, and we will report separately on the composition analysis and statistical confidence.

The complexity of this spectrum exceeds that of any other petroleum sample analyzed with our 9.4 T FTICR instrument for several reasons. First, positive-ion APPI produces both protonated and radical cations and many species are observed in both forms in a given spectrum. Second, the *m*/*z* range observed in this spectrum is nearly twice as wide (and extends to higher *m*/*z*) than is typically observed with our 9.4 T system. Third, the resolving power is ~30% higher than that of our standard petroleum measurement at 9.4 T (3.6 s time-domain signal duration at 9.4 T vs 3 s at 14.5 T). For the data shown here, the signal did not damp completely to zero during the acquisition period. Therefore, the resolving power achievable for a mixture of this complexity likely exceeds 1 000 000 (*m*/*z* 400) with the present 14.5 T instrument.

Ion Storage Capacity. With commercial 7 T LTQ FT systems, ions are stored in and transferred from the linear quadrupole trap to the ICR cell. The present 14.5 T instrument provides for a second mode of external ion accumulation in which ions are mass selected (or not) in the LTQ and transferred to an adjacent octopole equipped with wire ion extraction electrodes. This process may be repeated for numerous cycles before the entire octopole ion population is transferred to the ICR cell. That mode of operation is particularly useful for in-cell MS^n (e.g., ECD[34] or IRMPD[41]) and/or petroleum analysis, as illustrated by Figure 5.

Figure 6 illustrates a 2−3-fold improvement in ion storage capacity for LTQ selected ions (β casomorphin$^+$, ~864 Da) ejected from the wired octopole rather than those selected, stored, and transferred from the LTQ directly. Note that the same *total* ion number is selected/accumulated in all three experiments. Because ion storage in the LTQ is linear up to ~5 M charges, it is beneficial to instead transfer multiple smaller ion populations to the octopole, for which the charge capacity is ~10 M. Moreover, the axial electric field created by applying a dc potential (12 V in this case) to the wire extraction electrodes improves the ICR signal-to-noise ratio by ~50% relative to LTQ storage/transfer (i.e., compare LTQ storage (Figure 6, bottom) to a single octopole fill (Figure 6, middle). The axial field gradient is critical for efficient ion transfer when that octopole is used for selected ion accumulation. Ion trapping is achieved through collisional cooling with residual helium that escapes from the linear trap. Additional background gas (helium or nitrogen) may also be admitted to the wired storage octopole to facilitate noncovalent adduct dissociation for analysis of large biomolecules and provides an additional S/N improvement of 20−60% (data not shown) at target ion numbers less than 10 M. A similar sensitivity improvement is achieved by octopole ion storage/transfer for fragment ions produced by CAD in the linear ion trap and detected by FTICR. The use of several cycles of octopole ion storage alleviates the discrepancy between optimum ion number for CAD in the linear trap and that for FTICR detection. For broadband measurements, the applied axial electric field gradient can potentially reduce the observed *m*/*z* range due to increased time-of-flight dispersion between ions of different *m*/*z*. The extent of reduction in the observed *m*/*z* range depends on the voltage applied to the wires and sample composition and is negligible for applied voltages less than 5 V.

Ion transfer from the LTQ to the wired octopole adds 45 ms/cycle for broadband operation and 130 ms/cycle for selected ion/MS^n. Therefore, octopole ion storage is invoked only if a large ion population is needed for ICR analysis and the duty cycle is of

(41) Little, D. P.; Speir, J. P.; Senko, M. W.; O'Connor, P. B.; McLafferty, F. W. *Anal. Chem.* **1994**, *66* (18), 2809–15.

less concern (e.g., petroleum and/or structural characterization which requires internal ECD, IRMPD, or external CAD with FTICR product ion detection). For LC−MS, LTQ ion storage and transfer provides an adequate ion number and is preferred so as to maximize acquisition rate.

CONCLUSION

The 14.5 T LTQ FTICR mass spectrometer data exhibit the predicted increases in resolving power, mass accuracy, scan rate, and dynamic range for biological LC−MS and petroleum analyses compared to lower field systems. Automatic gain control complements high magnetic field strength to provide unprecedented mass accuracy for spectra collected at high mass resolving power and scan rate, a feature that is particularly powerful for LC MS analyses. High dynamic range and resolving power enable observation of a broader range and increased number of individual components present in complex petroleum samples than observed at lower field strength. An octopole ion trap installed between the linear quadrupole ion trap and the ICR cell increases the number of ions that can be delivered to the ICR cell and improves performance for petroleum analysis and tandem mass spectrometry.

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