

VOLUME 29 NUMBER 7
ACCOUNTS
of
**CHEMICAL
RESEARCH**®

JULY 1996

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Ion Cyclotron Resonance and Nuclear Magnetic Resonance Spectroscopies: Magnetic Partners for Elucidation of Molecular Structure and Reactivity

ALAN G. MARSHALL

Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310

Received September 19, 1995

Introduction

Mass spectrometry and nuclear magnetic resonance (NMR) are two of the most versatile and informative tools for investigation of molecular structure, bonding, and reactivity, and are usually regarded as conceptually separate disciplines. However, the highest-performance and most versatile type of mass spectrometry, namely, Fourier transform ion cyclotron resonance (FT-ICR), shares with NMR a broad range of conceptual, physical, experimental, and data reduction characteristics. In this paper, the fundamental conceptual homologies between FT-ICR and FT-NMR (mainly high-resolution liquid state) are exposed and explored to provide a deeper understanding of the highly parallel ongoing development and applications of the two techniques. The discussion develops from Table 1, followed by brief mention of historical parallels.

Chemical Motivation for NMR and ICR Analysis

When a sufficient amount (typically a few milligrams) of pure sample is available, FT-NMR is

Alan G. Marshall was born in Bluffton, OH, and earned his B.A. from Northwestern University in 1965 and Ph.D. with John Baldeschwieler in 1970 from Stanford University. He joined the faculty of the University of British Columbia in 1969, moved to The Ohio State University in 1980, and is now Professor of Chemistry at Florida State University and Director of the Ion Cyclotron Resonance Program at the National High Magnetic Field Laboratory in Tallahassee. He was co-inventor (with M. B. Comisarow) of FT-ICR mass spectrometry in 1973, and continues to develop new FT-ICR techniques and applications. Marshall has published more than 200 papers on ICR and magnetic resonance spectroscopies. His recent recognitions include Fellow of American Physical Society, Fellow of American Association for the Advancement of Science, American Chemical Society Award in Chemical Instrumentation, Eastern Analytical Symposium Award in the Fields of Analytical Chemistry, and American Chemical Society Field-Franklin Award.

unsurpassed in chemical information content: the number and types of chemical linkages from the Larmor frequency (chemical shift), bond angles from scalar (J) couplings, internuclear distances from dipole-dipole couplings, and bond strength and orientation (from quadrupolar couplings), leading to determination of complete three-dimensional structures of aqueous proteins up to 20 000 Da from NMR alone. Literally dozens of monographs describe FT-NMR techniques, and FT-NMR sales currently exceed one spectrometer per day!

For complex mixtures and/or much smaller sample amounts, mass spectrometry offers the richest available information: more than 50 000 mass spectrometers have been installed worldwide. In particular, FT-ICR mass spectrometry is the most versatile technique for identifying and quantifying gas-phase ion-molecule reaction pathways, kinetics, equilibria, and energetics in the absence of solvent, as described in more than 50 review articles (a few of the most recent of which are cited here¹⁻⁶), 3 journal special issues,⁷⁻⁹ and 3 books¹⁰⁻¹² over the past 10 years. In addition, Fourier transform ICR mass spectrometry has the following *analytically* important features:

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speed (~1 s per spectrum); ultrahigh mass resolution and ultrahigh mass accuracy for analysis of mixtures and polymers; low-volatility samples (10^{-9} Torr for electron ionization, and near zero for laser desorption/ionization or electrospray ionization); ultrasensitive detection (to a few attomoles of neutrals, or a single multiply-charged ion), tandem mass spectrometry with a single spectrometer, including two-dimensional FT/FT-ICR MS; positive and/or negative ions, simultaneously if necessary; multiple ion sources; peptide, nucleic acid, and oligosaccharide molecular weight and sequencing; and single-molecule detection up to 10^8 Da.¹³ The installed base of FT-ICR mass spectrometers approaches 200 worldwide, and is growing rapidly.

Signal Excitation and Detection

Natural Circular Motion. The basis for magnetic resonance and ion cyclotron resonance is Larmor precession of an individual magnetic moment or rotation of a single charged particle about a static magnetic field, $\mathbf{B}_0 = B_0\mathbf{k}$. The sense of the rotation is determined by the sign of the magnetogyric ratio (positive in NMR) or the sign of the ion charge (ICR). The natural rotation frequency, ω_{Larmor} or ω_c , is directly proportional to B_0 ; the proportionality constant reflects either the nearby electron distribution (from the "chemical shift" of the magnetogyric ratio, γ) or the charge-to-mass ratio, q/m . Larmor frequencies range from ~60 to 600 MHz for common magnetic nuclei (^1H , ^{13}C , ^{15}N , ^{31}P , ^{17}O , etc.) at 14 T, whereas ICR frequencies range from a few kilohertz to a few megahertz for ions of chemical interest ($12 \leq m/z \leq 100\,000$ at 7 T, where m is the ion mass (u) and z is the number of elementary charges per ion). Both experiments share much common radiofrequency hardware (frequency synthesizers, amplifiers, timing pulses, switches, filters, mixers, etc.) and circuitry.

To a first approximation, the peak width at half-maximum peak height, $\Delta\omega_{50\%}$ (rad s^{-1}), is independent of B_0 ; therefore, the frequency resolving power, $\omega/\Delta\omega_{50\%}$, increases linearly with applied magnetic field in either experiment. (In the cyclotron, the mass-to-charge ratio, m/q , varies inversely with frequency, but it is readily shown that the mass resolving power, $m/\Delta m_{50\%}$, is the same as the frequency resolving power.^{14,15})

Sample Containment. Containment of an NMR sample is trivial: one need simply enclose the sample in a rigid container which does not contain magnetic nuclei of interest. With a few exceptions (e.g., ^{23}Al ,

^{29}Si), that is not a problem. Containment of gas-phase ions for ICR is much more problematic. Although a static axial magnetic field confines ions (by virtue of their cyclotron motion) from escaping in the xy plane, ions are free to move in the $\pm z$ directions. It is therefore necessary to add an axial restoring force to confine ions from escaping in the $\pm z$ directions. Placement of two equally charged "end caps" at $\pm z_0$ produces an approximately quadratic axial electrostatic potential which in turn provides a linear axial restoring force, leading to simple harmonic axial oscillation (typically a few kilohertz) between the end caps. However, Laplace's equation requires that such a potential must be balanced by a quadratic radial potential "hill" which slopes quadratically downward with increasing radius, leading to (a) generation of a third slow (typically a few hundred hertz) "magnetron" rotational motion of ions due to the $\mathbf{E}(\mathbf{r}) \times \mathbf{B}_0$ force, where $\mathbf{E}(\mathbf{r})$ is the radial electrostatic field,¹⁶ (b) radial outward diffusion as ions roll down the radial potential hill due to collisions with neutrals,¹⁷ and (c) a small downward and correctable¹⁸ shift in the observed ICR frequency. Except for very massive ions, the ICR frequency is much larger than the axial oscillation or magnetron rotation frequencies.

Isotopic Abundance. NMR is of course possible only for those nuclei which have a magnetic dipole moment, and the NMR signal is therefore much weaker for species of low isotopic abundance (e.g., ^{13}C , ^{15}N) than for 100%-abundant magnetic isotopes (e.g., ^1H , ^{31}P). In mass spectrometry, on the other hand, one detects *all* isotopic combinations in proportion to their abundances (determined by binomial elemental isotopic distributions). Thus, although ^{13}C has a relative abundance of only ~1% compared to ^{12}C , a protein with (say) 500 carbons will actually exhibit ~ [500!/(1! 499!)] (0.01) = (500)(0.01) = 5 times as many $^{13}\text{C}^{12}\text{C}_{499}$ species as $^{12}\text{C}_{500}$ species.

Linearly-Polarized Excitation/Detection of Circular Motion. Although both NMR and ICR are based on *circular* motion, both kinds of motion are typically stimulated (excited) by applying a radiofrequency *linearly*-polarized dipolar excitation magnetic field (NMR) or voltage (ICR). One may think of the linearly-polarized field as the sum of two counter-rotating fields,¹¹ one of which rotates in the same sense as the magnetic moment (or ion) and the other in the opposite sense. Except when the Larmor (or ICR) frequency is very low, the opposite-sense rotating field has a negligible effect, and NMR or ICR motion is excited when the linearly-polarized excitation rf frequency matches (is "resonant" with) the Larmor (or ICR) frequency. (The opposite-sense rotating field situation results in various minor effects in NMR¹⁹ and ICR.²⁰) Truly circularly-polarized excitation may be produced by applying two rf signals that differ in phase by 90° (as by orthogonal pairs of electrodes in ICR). In ICR, circularly-polarized excitation makes it possible to distinguish between positive and nega-

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Table 1. Conceptual, Physical, and Experimental Analogies between Magnetic Resonance and Ion Cyclotron Resonance Spectroscopies

process or concept	manifestation in magnetic resonance	manifestation in ion cyclotron resonance
circular motion due to magnetic field	ω_{Larmor} : Larmor precession of magnetic moment	ω_c : cyclotron rotation of ion
sense of rotation	magnetogyric ratio sign	charge sign
sample containment	tube	electrostatic trapping potential
isotopes	signal observed according to isotopic abundance of magnetic nuclei only	signal observed according to isotopic abundance
linearly-polarized excitation/detection of circular motion	rf magnetic field (from rf current in one or more coils)	rf electric field (from rf voltage to one or more pairs of electrodes)
transducer(s) to convert excited circular motion into an oscillating electrical signal	single or crossed inductor coil(s); single-phase or quadrature detection	one or more pairs of opposed capacitor electrodes; single-phase or quadrature detection
signal amplitude proportional to \rightarrow	spin state population difference, which is proportional to B_0	total number of ions and ion cyclotron radius (i.e., signal is independent of B_0)
dynamic range	$\sim 10^6$ (with solvent-elimination tricks)	$\sim 10^6$ (with selective ejection tricks)
broadband excitation	frequency-sweep (chirp) excitation; tailored excitation	frequency-sweep (chirp) excitation; stored waveform inverse Fourier transform (SWIFT) excitation
linearity: response amplitude varies linearly with excitation amplitude	true only for small tip angle ($\sin \theta \approx \theta$)	dipolar excitation/response is highly linear, but quadrupolar excitation/response is linear only for small tip angle
sidebands due to spatially inhomogeneous time-dependent fields	"spinning" sidebands at integral multiples of sample spinning frequency	sidebands at small integral multiples of axial and/or magnetron frequency
high-resolution tunable-bandwidth detection	double- (super) heterodyne detection	single heterodyne for high-resolution or direct mode (i.e., no heterodyne) for broadband detection
phase cycling	many versions at multiple phase angles	only \pm (i.e., 180°) phase cycling currently in use
sample spinning to reduce spatial inhomogeneity in B_0	sample container is spun at 20–17000 Hz	sample spins and oscillates by itself at 100 Hz to ~ 1 MHz (cyclotron, magnetron, axial motions)
shimming to reduce static inhomogeneity	B_0 shimming with multiple correction coils	E_0 shimming with multiply segmented electrodes
shimming to reduce rf inhomogeneity	B_1 shimming (e.g., with birdcage coil)	E_1 shimming with multiply segmented electrodes
multiple remeasurement	sample relaxes by itself to thermal equilibrium between measurements; spin-echo	create new sample for each measurement, or force relaxation between measurements
unequal initial signals for repeated measurements	t_1 noise	ion number fluctuation noise
classical equations of motion	Bloch: time dependence of macroscopic magnetization components: M_x, M_y, M_z	Lorentz: time dependence of ion trajectory for dipolar excitation; Bloch: quadrupole excitation-interconversion between cyclotron, magnetron, and axial motions
detailed time-evolution formalism	quantum-mechanical time-independent perturbation theory for energy levels; semiclassical analysis of time-dependent problems	classical mechanics for ion trajectories; quantum treatment for single-ion low-temperature behavior or ion cooling phenomena
homogeneous ("lifetime" or "secular") relaxation; nonsecular relaxation	T_1 : spin state transitions due to B fluctuations at ω_{Larmor} and $2\omega_{\text{Larmor}}$; T_1 determined by sample	ion–molecule reactions or diffusion out of trap; ion radius damping due to collisions; T_1 adjustable by varying pressure
homogeneous nonsecular relaxation	T_2 : spin state transitions due to B fluctuations at 0, ω_{Larmor} , and $2\omega_{\text{Larmor}}$	nonreactive ion–neutral collisions: ion packet dephasing
extreme narrowing limit	$T_1 = T_2$: B fluctuations much faster than w_0	all ion–molecule collisions are reactive or all nonreactive heavy ions
inhomogeneous line-broadening	T_2^* : spatial inhomogeneity in B_0 (mainly) or B_1	spatial inhomogeneity in B_0 (minor), E_0, E_1 , or space charge from ions themselves; frequency drift
dissipation of signal into external detection circuit	radiation damping by resistive loss in detector coil	signal damping by resistive loss in detector electrode circuit
harmonic responses at multiples of the fundamental frequency	double (multiple) quantum transitions	doubly (multiply) charged ion; also harmonics due to nonlinear static and nonuniform rf electric fields
coherence	radiation (coherent); dipole–dipole coupling (incoherent); scalar coupling (coherent)	radiation (coherent); ion–neutral collisions and reactions (incoherent)
population transfer induced by resonant excitation	spin-tickling; double resonance; nuclear Overhauser effect	collision-induced dissociation by sustained off-resonance excitation (SORI); MS/MS
signal destruction or decoupling by \rightarrow population transfer by nonresonant excitation	saturation/strong resonant irradiation at w_0 off-resonance decoupling	ion ejection by strong resonant irradiation at ω_+ sustained off-resonance excitation for collision-induced dissociation
population exchange in absence of resonant excitation	chemical exchange	ion–molecule reactions, including electron, proton or ion transfer
laser-induced population transfer	laser chemically-induced dynamic nuclear polarization (CIDNP)	photodissociation, photodetachment
phase encodement and two-dimensional Hadamard and FT spectroscopy	Hadamard; two pulses with variable separation; 2D NOESY	Hadamard; two pulses with variable separation; direct SWIFT-based encodement; 2D SWIM
rotating coordinate frame	effective \mathbf{B} field	effective \mathbf{E} field
adiabatic rapid passage	population inversion without transitions	broadband quadrupole excitation-driven interconversion of cyclotron, magnetron, and axial motions
rotating frame behavior in the presence of a strong rf field	spin lock (change effective \mathbf{B} field in rotating frame to vary relaxation times)	ion lock (can double or eliminate effective \mathbf{B} field)
historical development	field sweep, frequency-sweep, pulsed FT; crossed coils, single coil	field sweep, pulsed FT; orthogonal electrode pair, single electrode configuration

tive ions of nearly the same m/z .²¹ Moreover, circularly-polarized ("quadrature") excitation is desirable in both NMR (and ICR) because it reduces the excita-

tion amplitude to half that for linearly-polarized excitation. Similarly, quadrature detection produces 2 times the signal and $\sqrt{2}$ times the noise, for a signal-to-noise improvement of $2/\sqrt{2} = \sqrt{2}$ compared to single-channel (linearly-polarized) detection.

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Detection Sensitivity. Just as a magnetic (or electric) field may be *generated* inside a transmitter coil (or between two opposed transmitter electrodes), the time-varying magnetic (or electric) field of a precessing magnetic moment (or cyclotron-rotating ion) may be *detected* from the current induced in a detector coil (or between two opposed detector electrodes). (In electrostatics, this correspondence is known as the "reciprocity" principle.²²) However, the strength of an NMR signal is proportional to the (very small) *difference* in population between nuclear spin states, whereas the ICR signal is proportional to the *total* number of ions.^{23–25} NMR excitation and detection are limited by the quantum-mechanically *finite* number of energy levels, so that the maximum signal strength is limited by the energy-level spacing, for which a single quantum transition energy is much smaller than $kT \approx 0.02$ eV, where k is the Boltzmann constant and T is absolute temperature. In ICR, on the other hand, the number of cyclotron energy levels is *infinite*, and a single ion may be excited to a cyclotron radius of several millimeters, corresponding to tens of electronvolts, or the equivalent of millions of absorbed individual cyclotron energy quanta. The detection limit for a single 1 s data acquisition for high-resolution proton NMR in a typical 0.3 mL sample is thus $\sim 10^{16}$ spins, whereas the corresponding FT-ICR detection limit is $\sim 10^2$ ions.²⁵ Finally, although intrinsic sensitivity for an undamped ICR signal for (the usual case of) capacitance-limited detection is independent of the mass-to-charge ratio,²⁴ the intrinsic NMR sensitivity for equal numbers of nuclei varies as the *cube* of the magnetogyric ratio, γ ,²⁶ because the magnetic moment is proportional to γ , the rate of change in the magnetic flux in a detector coil varies as ω_{Larmor} , which is proportional to γ , and the spin state Boltzmann population difference is proportional to γ .

Dynamic Range. At the other extreme (i.e., very large signals), the dynamic range can be especially large for ^1H NMR of aqueous biological samples because samples such as DNA (or proteins) cannot be put in D_2O without losing base-pair (or amide) protons. The resulting dynamic range (~ 100 M protons from H_2O vs 10^{-4} M protons from the sample) can easily exceed that of a 16-bit digitizer (65 536). Similarly, the "space charge" generated by more than $\sim 100\,000$ charges in an ICR ion trap sets an upper limit to the number of trapped ions, for a dynamic range of $\sim 10^3$ – 10^4 for a single ICR spectrum. In both cases, the dynamic range problem may be alleviated by eliminating the signal from the most abundant species: in NMR, by use of pulse sequences designed to suppress the solvent signal,²⁷ and, in ICR, by selective SWIFT ejection (see below) of the most abundant ions.²⁸

Broadband Excitation. Nuclei (or ions) of a *single*

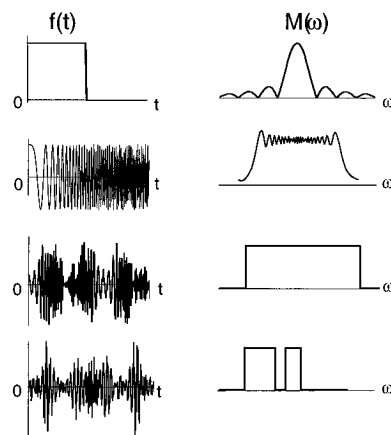


Figure 1. Time-domain ICR excitation waveforms (left) and their frequency-domain FT magnitude-mode spectra (right). Proceeding from top to bottom, rectangular (top-hat), frequency sweep (chirp), broadband SWIFT, windowed SWIFT (e.g., for broadband excitation of species of all but a narrow range of frequency).

Larmor (or cyclotron) frequency may be excited by single-frequency resonant irradiation. Broadband excitation can be more problematic, as shown in Figure 1. A "top-hat" single-frequency excitation of duration T (s) will distribute excitation over a bandwidth of $\sim 1/T$ Hz. For ^1H NMR (~ 10 ppm range of Larmor frequencies, or about 6000 Hz out of 600 MHz at $B_0 = 14$ T), a top-hat pulse lasting ~ 10 μs can thus excite the signals with acceptable uniformity over the desired spectral range. However, for other NMR nuclei or for ICR (where the range of cyclotron frequencies can exceed 1 MHz), a constant-amplitude linear (with time) frequency-sweep ("chirp") excitation spans a much wider frequency range.^{29,30} Even better, if the system is linear (see the next paragraph), then an optimally flat excitation vs frequency profile may be generated simply by inverse Fourier transformation to yield the corresponding time-domain excitation waveform. Known as "tailored" excitation in NMR³¹ or stored waveform inverse Fourier transform ("SWIFT") excitation in ICR,³² the inverse-FT method provides for flatter frequency-domain excitation³³ as well as greater mass selectivity for ion ejection in ICR.³⁴ Although occasionally employed in NMR and magnetic resonance imaging spectroscopy,³⁵ direct inverse FT excitation is less useful in NMR because NMR response varies nonlinearly with excitation amplitude or duration (see below).

Linearity. Generation and detection of spectral signals are particularly simple when the system is *linear*; i.e., the amplitude of the response is proportional to the amplitude of the excitation.¹¹ In NMR, the signal detected in a coil whose axis is perpendicular to \mathbf{B}_0 varies as $\sin(\gamma B_1 t)$, in which B_1 is the

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amplitude of the resonant dipolar rf magnetic field applied for a period, t , in a direction perpendicular to the z axis. $\gamma B_1 t$ represents the "tip" angle of the macroscopic magnetization away from the z axis. Therefore, NMR signal generation is linear only in the limit of small tip angle, $\sin(\gamma B_1 t) \approx \gamma B_1 t$. In ICR, on the other hand, dipolar excitation is highly linear even in an uncorrected cubic ion trap.³⁶

Sidebands. In both NMR and ICR, non-linearity can lead to signals at integral multiples (harmonics) of the desired resonance frequency and to "sideband" ("intermodulation distortion") signals spaced at some other characteristic frequency away from the desired resonance. For example, spinning the sample in NMR (see below) can generate "spinning sidebands" which flank the desired resonance at integral multiples of the spinning frequency, due to modulation of the magnetic field as the spins are carried back and forth between regions of different B_0 .³⁷ In ICR, sidebands flank the cyclotron resonance at integral multiples of the magnetron rotation and/or axial oscillation frequency, due to (weak) coupling between the cyclotron, magnetron, and axial modes of trapped ions.³⁸

Heterodyne Detection. Fourier data reduction (see below) requires sampling (digitizing) the time-domain signal at twice the highest detected signal ("Nyquist") frequency.¹¹ Thus, a typical time-domain data set size (say, 256K) sampled for 1 s limits the bandwidth to $\sim 10^5$ Hz. An ICR time-domain signal for ions of $m/z \geq 100$ or so may thus be sampled directly ("direct" mode). However, because Larmor frequencies can range to hundreds of megahertz, NMR detectors typically employ "heterodyne" detection to lower the signal frequency for detection. Heterodyne-mode detection (used also for ultrahigh-resolution FT-ICR) is like having a spectrometer with a "slit width" given by the (adjustable) bandwidth of a low-pass filter.¹¹

Signal Improvement

Phase-Cycling. A standard problem in rf spectroscopy is to distinguish a true signal from a spurious signal that happens to fall in the desired spectral range. Because only the true signal should result from coherent excitation, a common trick is to invert the time-domain excitation (i.e., 180° phase shift) for alternate data acquisitions, and alternately add and subtract the data to form an accumulated time-domain stored data set. Because the true signal inverts on alternate acquisitions, whereas spurious signals do not, the spurious signal should cancel out after an even number of acquisitions. Such "phase-cycling" is commonly employed in NMR (but less so in ICR, where it is more difficult to ensure an identical signal from one acquisition to the next). Elaborate phase-cycling schemes with multiple phase angles are employed in NMR to eliminate quadrature (see below) and other artifacts.^{26,39}

Sample Spinning To Reduce Spatial Inhomogeneity in B_0 . In NMR or ICR, the signal represents

a sum of fields generated by individual spins (or ions) distributed throughout a finite sample volume. If the static magnetic field amplitude varies from one part of the sample to another, then the corresponding Larmor (or cyclotron) frequency will also vary, leading to inhomogeneous line-broadening. In high-resolution NMR, the problem is solved by spinning the sample at a frequency of ~ 25 Hz for liquids, i.e., higher than the difference in NMR frequency between spatially different regions of the sample.⁴⁰ In ICR, the sample "spins itself" by virtue of ion cyclotron motion (a few kilohertz to a few megahertz) in a plane transverse to B_0 .⁴¹ Ions also average axial inhomogeneity in B_0 by axial oscillation (typically a few kilohertz) between the two end caps of the ICR ion trap. Thus, it is possible to obtain FT-ICR mass resolution of 0.1 ppm for ions in a magnet whose static inhomogeneity is ~ 10 ppm.⁴²

Shimming the Static Field. It is also desirable that the static magnetic (NMR) or electric (ICR) field be as spatially uniform as possible. In NMR, B_0 "shimming" is achieved by passing current through each of up to two dozen coils whose geometry produces correction fields with various spatial dependences, e.g., z , z^2 , z^3 , xy , yz , $x^2 - y^2$, etc. The shim coils with x and y contributions are adjusted for a nonspinning sample, and the z shims for a spinning sample. In ICR, the sample spins itself, so that interactive shimming of the electrostatic "trapping" potential is not feasible. Fortunately, it is possible to shim the electrostatic potential to near perfection by segmenting the ion trap and applying different static voltages to the different segments.⁴³

Shimming the rf Field. The rf excitation or detection field for NMR (or ICR) should be spatially uniform throughout the sample. In NMR, a simple Helmholtz coil suffices for small samples (as for high-resolution experiments in liquids), but a more complex (e.g., "birdcage") assembly is needed for magnetic resonance imaging applications for which field uniformity must extend over a bigger area.⁴⁴ In ICR, segmentation of a cubic ion trap into evenly spaced strips, with voltage varying linearly from one strip to the next, produces a near-perfect rf excitation electric field for both excitation and detection.⁴⁵

Multiple Remeasurement. In NMR, a single sample yields multiple repeated measurements because one need merely wait for a few T_1 periods (see below) for the macroscopic z magnetization to recover to its thermal equilibrium value. The signal-to-noise ratio of a repeated measurement increases as the square root of the number of co-added remeasured time-domain data sets. In ICR, remeasurement of the same ions becomes efficient when the ion mass is large compared to the neutrals with which it collides. After excitation and detection, the cyclotron radius collisionally damps rapidly to its thermal value (typically

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<1 mm), and ions may be reexcited and redetected.⁴⁶ However, ions diffuse continuously radially outward, so the remeasured signal decreases eventually due to an increase in the size of the ion cloud and ion radial loss. Therefore, ICR signal enhancement by signal averaging has until recently required generation of new ions for each measurement.

In both NMR and ICR, the signal may be "refocused" by suitable irradiation between measurements. The NMR "spin-echo" experiment employs an additional pulse to reverse the dephasing of ions and bring them back in phase to form an "echo".^{47,48} In ICR, quadrupolar excitation in the presence of ion-neutral collisions can reverse radial expansion of the ion cloud,⁴⁹ making possible remeasurement with >99% efficiency.⁵⁰⁻⁵²

t_1 Noise. In NMR, the signal varies slightly between successive measurements, due to variation in the excitation amplitude and/or phase. In one-dimensional experiments, it does not matter much, because many data sets are summed to yield the final spectrum. However, in two-dimensional FT-NMR, variation in the signal strength modulates the signal between various rows (the " t_1 " axis) of the two-dimensional data, leading to a noisy band through the final two-dimensional spectrum at the frequency of each strong peak.³⁹ In two-dimensional FT-ICR, a similar t_1 noise arises from variation in the ion signal due to fluctuation in the number of ions generated (separately) in successive experiments of the two-dimensional array.⁵³

Mathematical Description of the Experiment

Bloch and Lorentz Equations. Many NMR phenomena may be described by "phenomenological" Bloch equations,⁵⁴ which describe the time dependence of the *macroscopic* magnetic moment components M_x , M_y , and M_z as a function of \mathbf{M}_0 , \mathbf{B}_0 , \mathbf{B}_1 , T_1 , and T_2 .

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B}_0 + \gamma \mathbf{M} \times \mathbf{B}_1 - (M_x M_y, (\mathbf{M}_0 - M_z)) \cdot \left(\frac{\mathbf{i}}{T_2} + \frac{\mathbf{j}}{T_2} + \frac{\mathbf{k}}{T_1} \right) \quad (1)$$

γ is the magnetogyric ratio, \mathbf{M}_0 is the equilibrium macroscopic z magnetization, \mathbf{B}_0 is the applied static magnetic field, \mathbf{B}_1 is the applied rf magnetic field (usually in the xy plane), T_1 is the "longitudinal" or "spin-lattice" relaxation time which determines how rapidly M_z returns to equilibrium after a perturbation, and T_2 is the "transverse" or "spin-spin" relaxation time which determines how rapidly M_x or M_y returns to zero after a perturbation.

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The motion of an ion in an ICR ion trap is described by the Lorentz equation⁵¹

$$m \frac{d^2 r}{dt^2} = q \mathbf{v} \times \mathbf{B}_0 + q \mathbf{E}_0 + q \mathbf{E}_1 - \frac{\mathbf{v}}{\tau} \quad (2)$$

in which m and q are the ion mass and charge, r is the ion position, \mathbf{v} is the ion velocity, \mathbf{E}_0 and \mathbf{E}_1 are the static and rf electric fields inside the ion trap (see below), and τ is an exponential frictional damping time constant which represents ion-neutral collisions (reactive and nonreactive). The absence of a vector cross-product in the ICR rf excitation term, $q \mathbf{E}_1$, makes ICR *dipolar* excitation linear (unlike NMR). However, by use of Brown and Gabrielse's "V vectors" (i.e., normal mode amplitudes of the cyclotron and magnetron motions, analogous to raising and lowering operators in spin quantum mechanics),¹⁶ *quadrupolar* excitation in ICR may be cast in a form identical to the Bloch equations for particles of spin 1/2!⁵¹ Quadrupolar excitation in ICR is important because it affords a means for "shrink-wrapping" ions into a tight packet at the center of the ICR ion trap, thereby improving all aspects of FT-ICR performance (e.g., signal-to-noise ratio, mass resolving power, mass accuracy, ion remeasurement efficiency,⁵⁰ mass selectivity for MS/MS, etc.) by factors ranging from 2 to 1000.⁵¹

Detailed Time-Evolution Formalism. Both NMR and ICR are treated microscopically with respect to the effects of applied external fields, but statistically with respect to the effects of the surroundings. FT-NMR^{55,56} and FT-ICR⁵⁷ line shapes are well-described by the solutions of eqs 1 and 2. NMR transitions arise from Fourier components (at zero frequency, the Larmor frequency, or twice the Larmor frequency) of the power spectrum of the randomly fluctuating magnetic field from the surroundings. For ICR, the response of the ions to the applied static and rf electric fields is treated classically (first terms of eq 2) and the surroundings (i.e., ion-neutral collisions, ion-molecule reactions, resistive damping of the ICR signal, etc.) are represented by frictional damping (final term in eq 2).

Relaxation

Homogeneous Line-Broadening. In both FT-NMR and FT-ICR, the phase-coherent spin (NMR) or spatially-coherent ion (ICR) ensemble "relaxes" in two ways. First, relaxation may arise from a decrease in the signal *amplitude*--NMR spin population difference (NMR) or ion number or cyclotron radius (ICR). The time-domain signal decays exponentially (with time constant T_1), and the corresponding contribution to the frequency-domain line width is known as "lifetime-broadening". NMR lifetime broadening arises from transitions caused by magnetic field fluctuations at the Larmor frequency (or at twice the Larmor frequency).^{19,58} In ICR, lifetime-broadening results from loss in ion identity by reactive collisions with

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neutrals, diffusive loss of ions by neutralization at the trap boundary electrodes, or reduction in the ion cyclotron radius (as by collisions of a heavy ion with light neutrals).

A second type of homogeneous relaxation arises from loss in *phase coherence* and contributes to the time-domain exponential damping constant, T_2 (see Bloch equations). In NMR, such contributions to energy level transitions arise from magnetic field fluctuations at zero, Larmor, or twice the Larmor frequency.^{19,58} In ICR, dephasing of ions within a coherently orbiting ion packet can result from nonreactive ion-neutral collisions: the contribution to line width is $2/\tau$, where $1/\tau$ is simply the ion-neutral momentum-transfer collision frequency multiplied by the mass ratio, $m_{\text{neutral}}/(m_{\text{ion}} + m_{\text{neutral}})$.⁵⁹ Thus, high-mass ions colliding with low-mass neutrals can have a very narrow line width,⁵¹ leading to ultrahigh mass resolving power for ions up to 10^8 Da.¹³ An important difference between NMR and ICR is that the NMR T_1 is determined by the sample composition, whereas the ICR T_1 can be controlled by varying the pressure.²⁴

Extreme Narrowing Limit. This limit describes the situation in which $T_1 = T_2$. In NMR, extreme narrowing for all but very large molecules in liquids results when the highest frequency of fluctuation of the magnetic field from the surroundings is much higher than the Larmor frequency.^{19,58} In ICR, extreme narrowing corresponds to a situation for which either all ion-molecule collisions are reactive (so that an orbiting ion disappears from its packet on collision) or collisions are of heavy ions with light neutrals (so that collisions slow but do not deflect the ions.)⁵¹

Inhomogeneous Line-Broadening. Inhomogeneous broadening results from different resonant frequencies for identical particles. In NMR, inhomogeneous broadening arises mainly from a spatially inhomogeneous magnetic field throughout the sample, and such broadening is reduced by sample spinning, as noted above. In ICR, inhomogeneous broadening results mainly from deviations from the ideal quadrupolar electrostatic trapping potential in different parts of the ion trap.

Dissipation of the Signal into the External Detection Circuit. Both NMR and ICR are detected by the passage of electrical current through an external circuit. Thus, if the signal from the sample is sufficiently large, significant power will be dissipated through the equivalent resistance of that circuit. That power is drawn from the sample, with the effect that the observed signal damps exponentially with time. Such NMR "radiation damping"⁶⁰ contributes significantly to the line width for ^1H NMR in H_2O at $B \geq 10$ T or so. The similar problem in ICR is less severe, because if the number of coherently orbiting charges is large enough ($> 100\,000$) to produce the effect, ion-ion interactions dominate the line shape.

Harmonic Responses at Multiples of the Fundamental Frequency. Although the NMR quantum mechanical selection rule allows for only single-quantum transitions, use of multiple pulses for spin-spin coupled systems can generate multiple-quantum "coherences" (i.e., off-diagonal terms in the density matrix) which may be rendered observable by conver-

sion to single-quantum transitions.⁵⁶ In ICR, apart from the obvious multiple-frequency case of multiply-charged ions ($\omega_c = qB/m$), signals at harmonic-multiple frequencies may result from nonlinearity in the electrostatic trapping field, leading to odd-harmonic multiples (since even-harmonic contributions are zero by symmetry for ions whose cyclotron orbits fall on the central symmetry axis of the trap.⁶¹ Interestingly, harmonic-multiple signals may be generated by use of multiply-segmented detector electrodes, for improved mass resolution (because the peaks are farther apart) in some cases.⁶²⁻⁶⁴

Coherence. Interactions may be the same ("coherent") or different ("incoherent") for every member of an ensemble of spins (or ions). For example, the rf irradiation of spins (or ions) is coherent, because the wavelength of rf radiation is large compared to the size of the NMR (or ICR) sample. Alternatively, through-space dipole-dipole coupling in NMR and ion-molecule reactions in ICR affect spin (or ion) populations incoherently, because not every pair of magnetic dipoles is equally spaced or oriented and not every ion reacts at the same instant. The similarity between those NMR and ICR phenomena underlies the similarity of NMR NOESY and ICR two-dimensional MS/MS experiments to detect those couplings. However, isotropic through-bond scalar couplings in NMR are the same for each molecule in the sample, and there is no corresponding coupling in ICR; thus, there is no ICR analog to the NMR COSY experiment, which depends on coherent coupling between resonant species.

Population Transfer

Population Transfer Induced by Resonant Excitation. One of the most chemically useful features of NMR and ICR is the use of "double resonance"^{65,66} to probe "couplings" between species by irradiating at the resonant frequency of one or more species, and observing changes in the populations of other species coupled to the irradiated species. In NMR, one type of coupling is the through-space magnetic dipole-magnetic dipole interaction, whose energy varies as $1/r^3$, in which r is the distance between the two spins. The coupling is typically manifested in T_1 , which (because the effect shows up in second-order time-dependent perturbation theory) leads to a $(1/r^6)$ distance dependence. "Spin-tickling" denotes population changes induced by low-power irradiation of the target resonance. The so-called "nuclear Overhauser effect" describes the change in relative intensity of an NMR signal on irradiation of one of its coupled partners.

In ICR, the corresponding couplings are ion-molecule chemical reactions. Irradiation at the cyclotron frequency of ions of a particular m/z increases the cyclotron radius (and thus kinetic energy) of those

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ions, resulting in changes in the populations of any products of chemical reactions of the irradiated ion. By perturbing the populations of each of the "parent" ions in a sample, one can sort out ion-molecule reaction pathways and quantitate (i.e., kinetics, equilibria, energetics) complex families of ion-molecule reactions.⁶⁷

Signal Destruction or Decoupling by Application of a Strong Irradiating rf Field. In both NMR and ICR, it is often convenient to "uncouple" interactions to determine which species are coupled to which. For example, an NMR signal may be eliminated by sufficiently strong irradiation at its Larmor frequency, leading to "saturation" (e.g., equalization of populations of two states of a spin 1/2 system). Similarly, strong dipolar irradiation at the cyclotron frequency can drive an ion to a cyclotron orbital radius larger than the ion trap, so that ions collide with the trap sides and are "ejected". Conversely, continuous quadrupolar irradiation of ions of a particular m/z can hold them on-axis while ions of other m/z diffuse radially until they strike the trap sides.⁶⁸ Finally, NMR "decoupling" may be achieved without the need for complete saturation. One need simply induce rapid transitions of one nucleus, so that the other nucleus effectively undergoes rapid chemical exchange between its two coupled energy levels; if the exchange rate is faster than the difference in energy between the two states, the spectral splitting collapses into a single peak.⁶⁵

Population Transfer by Nonresonant Excitation. In both NMR and ICR, it is sometimes convenient to employ off-resonant excitation, because high-power decoupling (NMR) or ion ejection (ICR) can perturb nearby resonances as well. In NMR, off-resonance irradiation of (say) protons gives a ¹³C spectrum in which multiplicities are preserved (e.g., identifying CH₂ vs CH₃), whereas the ¹³C peak magnitudes vary with the ¹H irradiation frequency offset and thus help to assign which ¹³C resonances are associated with which ¹H resonances.³⁹ In ICR, the corresponding experiment is known as "sustained off-resonance irradiation" (SORI),^{69,70} for periodic gentle heating of ions to induce fragmentation or reaction along relatively low-energy pathways.

Population Exchange in the Absence of Resonant Excitation. In NMR, the "time scale" for population change in the absence of applied radiation depends on whether the "exchange" between different sites is fast or slow compared to the difference in NMR parameters (Larmor frequency, scalar coupling constant, dipolar coupling constant, quadrupolar coupling constant, etc.) between the two sites. In the "slow" exchange limit, the resonance at each site is broadened by $(k_{\text{forward}} + k_{\text{reverse}})$ rad s⁻¹, in which k_{forward} and k_{reverse} are the rate constants for forward and reverse reactions. In the "fast" exchange limit, the NMR parameter of interest is simply the population-weighted average of the parameters at each site.⁷¹ In ICR, the width of a spectral peak is broadened by the inverse

pseudo-first-order rate constant (rad s⁻¹) for ion-molecule reactions (e.g., proton, electron, or ion exchange, reaction, fragmentation, etc.).⁵⁷

Laser-Induced Population Transfer. In NMR, a laser may be used to polarize the electron spins on small molecules which diffuse to transfer their polarization to spins on aromatic amino acids on the surface of a protein. Such "laser CIDNP" (chemically-induced dynamic nuclear polarization⁷²) experiments can effectively increase the nuclear spin polarization by a large factor (up to 50) to enhance the NMR signals from surface residues in a macromolecule. In ICR, a laser may change ion populations by fragmenting an ion by photodissociation, or by removing a negative charge by photodetachment. Photodissociation is especially useful for distinguishing between two or more ions of the same mass but different chemical structure, e.g., C₇H₈⁺ ions.⁷³

Phase Encodement and Two-Dimensional Spectroscopy. In both NMR⁶⁵ and ICR,⁷⁴ one can establish couplings between each of the resonances of interest from a series of (one-dimensional) experiments. In each experiment, a single resonance is irradiated, and its effect on all other resonances is determined from broadband excitation and detection and FT data reduction. It would clearly be more efficient to irradiate *all* of the possible resonances at once in a suitably encoded way, and then vary the code from one experiment to the next: for N linearly independent encodements, one can extract N possible couplings. This idea can be achieved in either of two ways.

First, if one knows in advance the Larmor (or cyclotron) frequency of each peak of interest, then each of a series of SWIFT waveforms can excite at all of the peak frequencies (with relative amplitude of +1 or 0 according to each row of a Hadamard code matrix), followed by FT analysis of each resulting spectrum. Decodement is achieved by a "fast Hadamard transform" similar to the "fast Fourier transform" (FFT). Although Hadamard encodement has been demonstrated in both NMR and ICR,⁷⁵ it is seldom used (except for magnetic resonance spatial imaging⁷⁶), because one must know the locations of all of the resonances in advance and because the excitation amplitude must be tuned accurately for optimal results.

A second method is based on phase encodement of resonances according to their Larmor (or ICR) frequencies. Phase encodement has been achieved in NMR⁵⁶ and ICR⁷⁷ by application of two identical pulses (single frequency or chirp) separated by a delay period that is incremented linearly from one experiment to the next, as in NOESY for NMR, or more directly in ICR⁵³ by use of a series of stored waveform ion modulation (SWIM) excitation waveforms.

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Rotating Coordinate Frame

Visualization of both NMR and ICR experiments is easier in a coordinate frame that rotates at the Larmor (or cyclotron) frequency. In NMR, the static \mathbf{B}_0 field vanishes in the rotating frame (i.e., if the magnetization is fixed in direction, no magnetic field can be present); addition of an rf \mathbf{B}_1 field that rotates in the lab frame then results in a fixed \mathbf{B}_1 field in the rotating frame, and the magnetization simply rotates about that (rotating) \mathbf{B}_1 field.¹⁹ In ICR, resonant ions in a rotating frame are fixed; addition of an rf \mathbf{E}_1 field that rotates in the lab frame results in outward radial straight-line ion motion.⁷⁸ In a frame rotating at the ion cyclotron frequency, off-resonance excitation in ICR makes ions travel in a circular path tangent to their starting point, with a radius that reflects the maximum kinetic energy periodically absorbed by the ions.⁷⁸

Adiabatic Rapid Passage. Consider the effect of irradiation at a frequency that sweeps rapidly from one side of the resonance to the other. In a frame rotating at the Larmor frequency, the "effective" field (i.e., the vector sum of the transverse component, \mathbf{B}_1 , and the axial field due to off-resonance component from \mathbf{B}_0) initially points along the positive z axis, and then rotates down through the xy plane, ending up pointing along the negative z axis.⁷⁹ If there are no transitions during this process, then the macroscopic magnetization, \mathbf{M}_0 , simply follows the effective magnetic field in the rotating frame, and ends up pointing along the negative z axis (i.e., population "inversion"). This method of producing a so-called "180° pulse" is relatively nonselective, since spins of all Larmor frequencies in the swept range will be inverted, but is experimentally simple.

In ICR, a formally identical process occurs for azimuthal quadrupolar (rather than dipolar) excitation, and the 180° pulse corresponds to complete interconversion between magnetron and cyclotron motion.^{51,80} Ions initially off-axis (i.e., with large magnetron amplitude) are brought on-axis with large (but rapidly collisionally damped) cyclotron amplitude. This method thus offers a nonselective (but simple) means for broadband ion axialization.

Rotating Frame Behavior in the Presence of a Strong Irradiating rf Field. A particularly interesting situation in NMR and ICR happens when spins or ions are first excited (90° pulse or large cyclotron radius) and the phase of the rf excitation is then changed by 90°. In the rotating frame, the spins (or ions) are effectively "locked" along the (rotating) magnetic (or electric) effective field. In NMR, the method allows for measurement of relaxation in a rotating frame magnetic field that can vary over a wide range, thereby exposing relaxation processes of widely differing rates.⁸¹ In ICR,⁸² the effective magnetic field may be zeroed (for near-instantaneous selective ion ejection) or doubled.

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Fourier Transform Spectroscopy

Here the correspondence between NMR and ICR is so complete that it is hardly necessary to distinguish between the two. In fact, many of the basic Fourier transform spectroscopy techniques of NMR and ICR were adapted from prior FT-interferometry developments.¹¹ Common aspects include zero-filling (to improve digital resolution),^{83–85} foldover,⁸⁶ apodization (windowing) to enhance the spectral line shape and/or signal-to-noise ratio,¹¹ quadrature excitation and detection,^{87–90} phase correction,^{11,91} dispersion-*vs*-absorption line shape analysis,⁹² "leakage" (due to truncation of the time-domain data before the signal has decayed fully to zero),¹¹ FFT data reduction,¹¹ non-FT data reduction (linear prediction, maximum entropy, etc.),¹¹ correlation spectroscopy,^{93,94} oversampling,^{95,96} deconvolution,^{11,97} pseudorandom noise ("stochastic") excitation,^{98,99} and many others.

Historical Parallels

ICR and FT-ICR mass spectrometries were originally conceived and have in many respects continued to develop by analogy to NMR and FT-NMR spectroscopies. Much of the early development of ICR mass spectrometry took place at Stanford University in the laboratory of John Baldeschwieler, whose prior research centered on NMR spectroscopy. Later, FT-ICR mass spectrometry was developed at the University of British Columbia by two researchers (Melvin B. Comisarow and Alan G. Marshall), both of whose Ph.D. research centered on NMR. Interestingly, three developments which occurred separately in NMR (spinning the sample,⁴⁰ frequency sweep rather than magnetic field sweep, and the multichannel advantage of pulsed FT spectroscopy⁵⁵) effectively occurred simultaneously with the introduction of FT-ICR in 1974.¹⁰⁰

In both NMR and ICR, the experiment was performed first by detecting power absorption at fixed frequency as the magnetic field was swept through resonance,^{101–103} and later by FT of the response at

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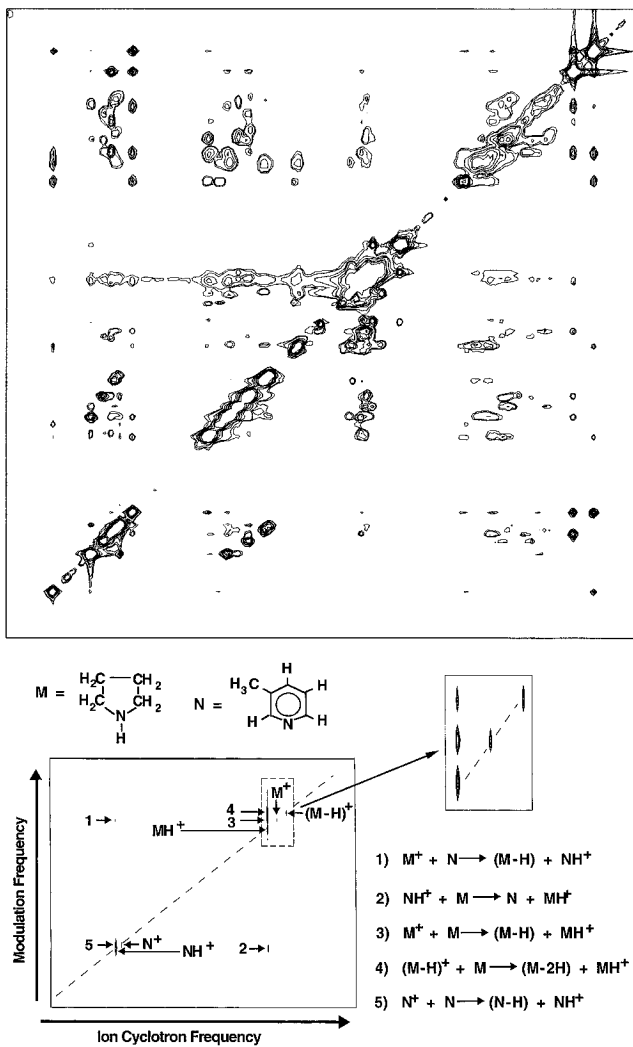


Figure 2. Two-dimensional NMR (top) and ICR (bottom) spectra. The NMR data (kindly provided by C. Zwalen, S. Vincent, and G. Bodenhausen) derive from phase encodement based on two pulses separated by a variable time delay to yield a phased "NOESY" spectrum (with water-flip back¹⁰⁹ and WATERGATE¹¹⁰ for water suppression) of "Dickerson's" self-complementary palindromic base-paired duplex B-DNA dodecamer¹¹¹ d(CGCGAATTCGCG)₂ in ~95% D₂O. Each frequency (chemical shift) axis runs from 1 to 9 ppm. The ICR data derive from phase encodement based on stored-waveform ion modulation (SWIM) excitation to yield (unphased) isomagnitude contours: five ion-molecule proton-transfer reactions of pyrrolidine and 3-methylpyridine may be identified from the off-diagonal peaks in this spectrum.⁵³ Each of these two-dimensional experiments thus represents a technique that is feasible (but not yet attempted) for the other type of spectroscopy.

fixed magnetic field to a short pulsed excitation.^{29,55,104} The experiment was originally conducted with one coil (or one pair of opposed electrodes) to excite the signal

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and a second orthogonal coil (or pair of opposed electrodes) for detection,^{54,105} and later with a single coil¹⁰⁶ (or detector electrode assembly⁴⁵). Excitation and detection were first conducted in single-phase mode, and later in quadrature mode.⁸⁷⁻⁹⁰ Future parallel evolution and cross-fertilization can be expected in areas such as two-dimensional spectroscopy (see below).

Conclusion

Progress in science is usually made either by identifying and exploring differences between similar concepts or techniques or by perceiving and exploiting similarities between superficially different problems. In magnetic resonance, the second approach has clearly been the more productive, accelerating the development of ICR and FT-ICR mass spectrometries by analogy and correspondence to closely related phenomena in nuclear magnetic resonance, which in turn borrowed heavily from prior developments in optical spectroscopy and interferometry. A number of NMR techniques remain to be exploited in FT-ICR, such as phased one- and two-dimensional spectra (Figure 2, top), quadrature phase-cycling, and time-shared excitation/detection, whereas some ICR techniques may apply to NMR (e.g., stored-waveform excitation at a small tip angle for 2D FT/FT NMR), as shown in Figure 2, bottom.

Although the uses of FT-NMR for determination of the three-dimensional configuration(s) of large (especially biological) molecules are well-known to most chemists, future applications for FT-ICR mass spectrometry are perhaps less widely recognized. For example, FT-ICR's high resolution should make possible the identification of an order of magnitude more components in complex mixtures (such as crude oil distillates).¹⁰⁷ Attomole sensitivity from multiple re-measurements¹⁰⁸ could lead to characterization of the chemical constituents of a single biological cell. The high speed and sensitivity of FT multiplex detection combined with HPLC separation could replace two-dimensional gel electrophoresis for separation and identification of peptides and proteins. Finally, various FT-ICR techniques are currently being developed to establish the three-dimensional configuration(s) of "naked" gas-phase biological macromolecular ions with no solvent present: such information should prove critical in testing theoretical models of protein structure and folding, as well as determining the role of water in protein structure.

Many of the ideas in this Account were stimulated by the author's highly talented and creative co-workers. The author wishes to acknowledge particularly L. Chen, M. B. Comisarow, M. V. Gorshkov, P. B. Grosshans, S. Guan, Z. Liang, J. Meier, T. L. Ricca, D. C. Roe, L. Schweikhard, F. R. Verdun, M. Wang, T.-C. L. Wang, L. G. Werbelow, and X. Xiang. Helpful suggestions and revisions were provided by S. Vincent and S. Guan. The author also thanks C. Zwalen, S. Vincent, and G. Bodenhausen for providing Figure 2 (top). This work was supported by the NSF (Grant CHE-93-22824), the NIH (Grant GM-31683), and The National High Magnetic Field Laboratory at Florida State University.

AR9501481