A Dynamic Ion Cooling Technique for FTICR Mass Spectrometry

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A fast dynamic ion cooling technique based upon the adiabatic invariant phenomenon for Fourier transform ion cyclotron resonance mass spectrometry (FTICR) is presented. The method cools ions in the FTICR trap more efficiently, within a few hundred milliseconds without the use of a buffer gas, and results in a substantial signal enhancement. All performance aspects of the FTICR spectrum, e.g., peak intensities, mass resolution, and mass accuracy, improve significantly compared with cooling based on ion–ion interactions. The method may be useful in biological applications of FTICR, such as in proteomic studies involving extended on-line liquid chromatography (LC) separations, in which both the duty cycle and mass accuracy are crucially important. (J Am Soc Mass Spectrom 2001, 12, 1169–1173) © 2001 American Society for Mass Spectrometry

The cooling of ion translation motion in FTICR-MS [1, 2] is desirable for many reasons, the most important of which are: (1) detection of ions at much lower trapping potentials and, therefore, reduced effects of electric field inhomogeneity; (2) reduction of the ion axial, spatial, and energy distribution and, therefore, the trapping and detecting of ions in the most homogeneous magnetic field region; and (3) an increased intensity for the FTICR signal as the ions are less subject to axial losses during dipolar excitation of their cyclotron motion. The problem of ion cooling becomes even more important when the ions are generated externally and introduced into the FTICR trap in pulses. If the excitation/detection event in the experimental sequence immediately follows ion capture, the acquired FTICR signal will exhibit reduced mass resolution, peak shape, and intensity because of broad ion spatial and energy distribution. To overcome this problem, it is a common practice to use a so-called ion cooling event immediately following ion trapping. In its simplest form, the ion cooling involves a delay during which ions are allowed to interact via Coulomb forces which results in a decrease of the ion cloud temperature (and ion axial oscillation amplitudes) which are due to the loss of energetic ions from the trap. This evaporative cooling process, and its variants under different names, is widely used in FTICR (a comprehensive most recent review of all the methodological aspects of FTICR can be found in [3]). Figure 1 shows an example of how evaporative (or ion–ion) cooling affects signal quality (e.g., relative peak intensity). A more than a 100-fold increase in a peak's relative intensity (melittin +3 charge state), and a correspondingly dramatic change in mass resolution, were observed. The drawback of evaporative ion cooling is the long time required which makes it unsuitable for many applications (e.g., during on-line capillary LC separations used in proteome studies [4, 5]). A common method for increasing the duty cycle involves injection of a buffer gas [6, 7]. In Figure 1, it can be seen that the performance achievable with tens of seconds of evaporative cooling can be obtained in just a few seconds using buffer gas introduction. However, the price for the increased duty cycle is an increased gas load on the pumping system, higher background pressure, and lower mass spectrometric resolution unless a time delay is introduced for pressure to return to a level suitable for the desired measurement quality.

Clearly, there is a need for fast ion cooling without the use of a buffer gas, and several approaches have previously been discussed or demonstrated, notably, resistive [8], sympathetic [9, 10], and adiabatic ion cooling [11, 12]. The first two are not practical for FTICR applications (although, sympathetic cooling proved to be useful for analysis of negative ions with FTICR [10]). The effect of adiabatic cooling can be appreciated by a closer examination of the ion cloud energy. The ions arrive at the cell dispersed along the axis and dispersed in energy. At the appropriate time after ejection from

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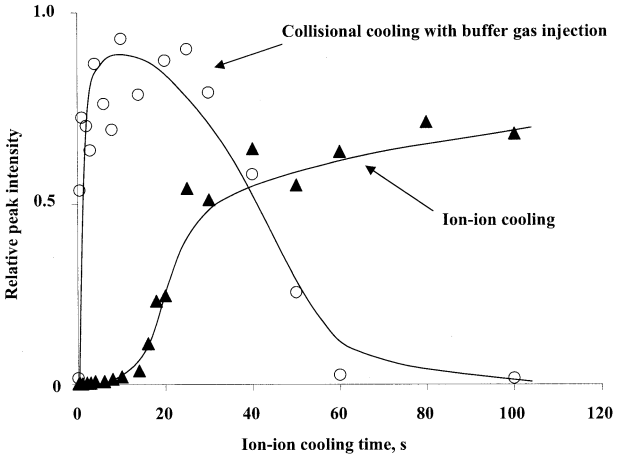

Figure 1. Dependence of relative peak intensity on the period of ion cooling after gated trapping. After the cooling period, the trapping potential was dropped to 0.5 V. Addition of the buffer gas greatly accelerates the cooling process, but results in more pronounced ion losses due to magnetron expansion. Melittin +3 charge state ions.

the external rf-trap, chosen to maximize the signal for some *m/z* range, the voltages on the end plates of the ICR cell are raised suddenly to a voltage sufficient to trap ions. This sudden increase of the electrostatic potential increases the average total energy of the trapped ions. The energy is initially unevenly distributed and gradually equilibrates. Simultaneously, the ions begin to cool by a slow loss of the most energetic ions. The voltages on the end plates are then lowered suddenly to the voltage to be used during the detection cycle. At this point ions with energies above the trapping voltage escape from the trap, further cooling the ion population. However, the fraction of the remaining ions having energies just below the trapping voltage oscillate over the whole region of the trap's axis. This motion limits achievable resolution and mass accuracy since some fraction of the ion population will be in the anharmonic regions of the trap.

Clearly, if the end cap voltages could be lowered in a manner that would confine the ions to a smaller region of the ICR trap, one would see improvements in the resolution and mass accuracy of measurements. It should be noted that one could start lowering the voltages sooner as the equilibration of energy need not be complete at the start of the voltage ramp. The principle of adiabatic invariance of the action integrals of a mechanical system is the basis for adiabatic ion cooling and suggests that such a dynamic lowering of the end cap voltages (at an appropriate rate) is feasible. There should be no confusion between evaporative mechanism of ion cooling and adiabatic expansion of the ion cloud. The latter results in a decrease in translational energy of all ions present in the FTICR trap through the adiabatic invariant phenomenon, not just the loss of the most energetic ions. In this work we demonstrate how to implement the non-buffer gas ion cooling in FTICR based on the adiabatic invariant phenomenon, which works in broad *m/z* range, and effectively cools all the ions within a relatively short period of time (fraction of a second).

A first order analysis of the method can be made based on the assumption that the ion axial oscillations are periodic motions characterized by the trapping frequency, ω_{tr}. To the first approximation, the equation of ion axial (or z) motion is an equation of harmonic oscillator.

$$z'' + \omega_{tr}^2 z = 0 \tag{1}$$

In eq 1 the trapping frequency is a parameter which characterizes the properties of ion axial oscillations in the trapping electric field. If this parameter is constant, the system is considered to be conservative (the ion axial energy stays the same over the period of ions oscillations). However, this parameter for the system may vary because of the change in the external field which makes the system non-conservative. If the parameter changes slowly, the corresponding slow change in average energy, $\langle E \rangle_T$, (over the much faster period of trapping oscillations) will be proportional to the change of the parameter. In other words, there is a value that is a function of both energy and ω_{tr}^2, which remains constant. It is known from theoretical mechanics [13] that the action integral, I, is such a function which for cyclic variables in the phase plane of a conjugate momentum, p, and its coordinate, ξ, is defined by the trajectory integral, $I = 1/2\pi \oint p d\xi$. For the case of an harmonic oscillator (i.e., ion axial oscillations), the integration is straightforward and results in a simple relationship between energy and frequency.

$$\mathrm{I} = \frac{\langle \mathrm{E} \rangle_T}{\omega_{tr}} \tag{2}$$

The adiabatic invariance of I means that when the frequency of ion axial oscillations changes slowly the value of I is unchanged, so that the ion axial energy changes proportionally with frequency.

The equation for the frequency of axial oscillations is

$$\omega_{tr} = \sqrt{\frac{\beta q \mathrm{V}_{tr}}{ma^2}} \tag{3}$$

in which V_{tr} is the trapping potential, β is the geometry factor of the trap of particular geometry (e.g., 2.77 or 2.84 for cubic and cylindrical traps, respectively), and a is the size of the trap. Therefore, we conclude that by a slow change of the trapping potentials, one can realize the adiabatic invariance conditions and change the ion axial energy. The rate of this change should satisfy the following inequality.

$$\mathrm{T}\frac{\mathrm{d}\omega_{tr}^2}{\mathrm{d}t} \ll \omega_{tr}^2 \tag{4}$$

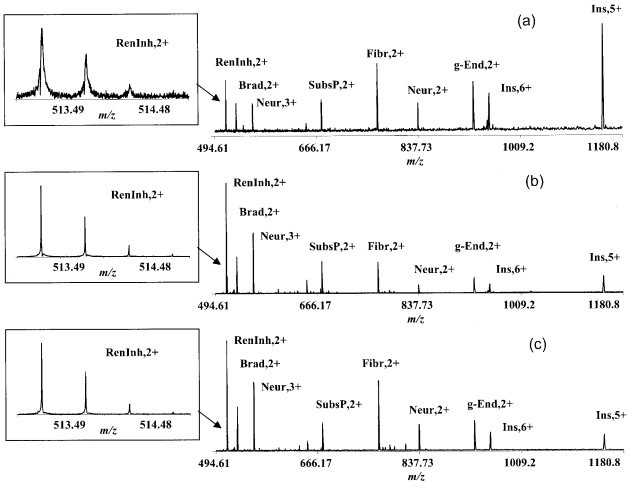

Figure 2. Results of experiments with mixture of several peptides and insulin: (**a**) 300 ms ion–ion cooling; (**b**) 10 s ion–ion cooling; and (**c**) 300 ms dynamic ion cooling. In all cases the experimental conditions at which the ions were produced, accumulated, and transferred into the trap were the same. The external accumulation period was 20 ms, transfer time was 2.5 ms, and trapping potentials were raised to 4 V during gated trapping.

meaning that the parameter, ω_{tr}^2, changes negligibly over the period of an oscillation. From eq 3 the requirement (eq 4) can be written as

$$\frac{dV_{tr}}{dt} \ll \frac{1}{2\pi}\sqrt{\frac{\beta q}{ma^2}}V_{tr}^{3/2} \tag{5}$$

The inequality (eq 5) tells us that higher *m/z* ions and larger FTICR traps will require longer cooling periods. We can thus estimate how quickly the ions can be cooled for some practical applications. For a 2-inch-size cubic FTICR trap, an upper *m/z* range of 1000, and a trapping potential decrease from 10 V to 0.5 V, to realize the adiabatic invariance requirement we have $dV_{tr}/dt \ll 6 \times 10^2$ V/s, in which the right side of the inequality corresponds to 0.5 V trapping potential. This estimate gives a rate of change in trapping potentials of less than ~100 V/s. Therefore, it appears possible to cool the ions by lowering the FTICR trapping potentials from 10 V to 0.5 V over a period of several hundred millisecond for the *m/z* range of most applications.

In this work we demonstrate experimentally broadband *m/z* ion cooling based on the adiabatic invariance phenomenon. Note that ion cooling for high performance FTICR measurements by means of stepwise decrease in trapping potential has been previously demonstrated, although it was more likely based on evaporative or ion cloud expansion mechanisms of ion cooling, and required longer cooling periods (up to 60 s in some instances) [14]. Thus, it is important to point out that the decrease in trapping potentials should be smooth in order to realize the adiabatic invariance. To distinguish the method discussed in this work from other adiabatic cooling methods, we refer to it as dynamic ion cooling (DIC) to indicate that the trapping potentials change continuously during the cooling process.

Experimental and Results

All experiments were performed using an 11.5 tesla FTICR mass spectrometer developed and constructed at Pacific Northwest National Laboratory. The instrument is controlled by an Odyssey (Finnigan, Madison, WI) data-station, equipped with an electrospray ion source and an elongated cylindrical open-ended FTICR trap [15]. A +2 kV voltage was applied to the electrospray ionization (ESI) emitter, and charged species were injected through a 500 μm diameter heated metal capillary maintained at 160 °C. The ions were accumulated in an external storage quadrupole and then ejected from the quadrupole into the FTICR trap through a rf-only quadrupole ion guide. Trapping potentials applied to the FTICR cell were synthesized by arbitrary waveform generator (DAQArb 5411, National Instruments Corporation, Austin, TX), which was controlled through the ICR-2LS software package [16] (upgraded to allow the synthesis of the dynamic trapping voltages). The optimal ions transfer time between accumulation quadrupole and FTICR trap was not alike for different *m/z* values and the total transfer time spread for the ions in the *m/z* range from 500 to 1200 was as long as 2 ms.

A comparison was made between two non-buffer gas cooling methods. In the first method, ions were cooled through ion–ion interactions during the time delay in which the trapping potentials remained constant and above the ions energy. In the dynamic ion cooling method, the trapping potentials were monotonically decreased immediately after the gated trapping event from potentials above the ions energy to potentials best suited for high resolution detection. The time of dynamic ion cooling was also varied from 10 ms to 500 ms, with 300 ms used in most experiments. One of the modifications to the method also evaluated in this work involved the rapid rise of trapping potential much higher (up to 100 V) than the ion energy immediately after the gated trapping event followed by the adiabatic decrease of the potential to a fraction of a volt for detection. We found that when the trap was operated without capacitive coupling, the maximum signal enhancement was achieved with a trapping potential increase to 15–20 V (any further effect from an increase in trapping potential was probably limited by other capacitance contributions to the circuit). With addition of the capacitive coupling this increase was limited to 4 to 5 V (i.e., just above the ion energy). A linear decrease in trapping potential was used for all results presented. Efforts to optimize the functional form of the adiabatic decrease in trapping potential are in progress, and initial results indicate that a linear decrease in trapping potential is most effective.

Figure 2a shows a spectrum for a peptide/protein mixture obtained with gated trapping followed by 300 ms ion–ion cooling. The transfer time was chosen to be

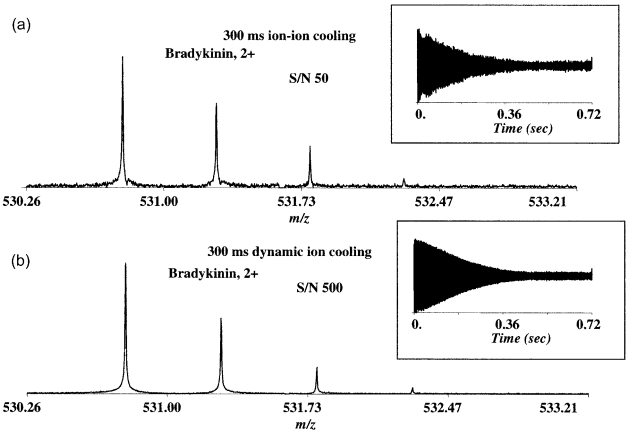

Figure 3. Demonstration of the effect of using the dynamic ion cooling technique on the FTICR signal from individual ions. Ion transfer time was optimized at 2 ms, which corresponded to the best gated trapping conditions for bradykinin +2 charge state ions. (**a**) 300 ms ion–ion cooling at trapping potential of 9 V; (**b**) 300 ms dynamic ion cooling with an initial trapping potential rise to 9 V. In both cases ions excitation and detection used trapping potential of 0.5 V.

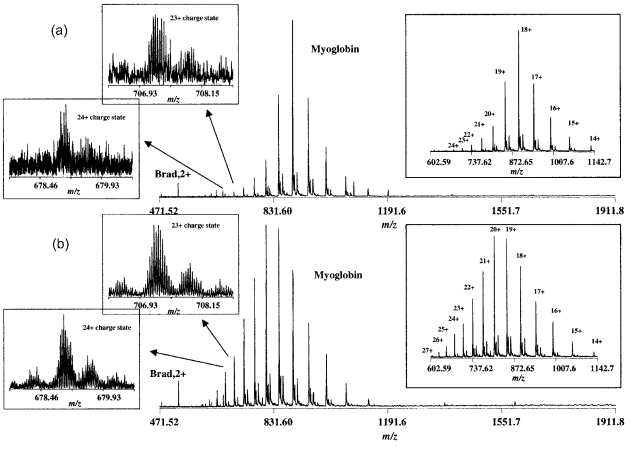

Figure 4. FTICR mass spectrum for ESI of a myoglobin solution. (**a**) 500 ms ion–ion cooling at 4 V trapping potential. Because the transfer time was optimized for the most abundant peak, the charge state distribution is biased toward this peak (+18 charge state ions). (**b**) 500 ms dynamic ion cooling (4 V to 0.5 V). Charge state distribution has shifted because of enhancement in signals from higher charge state ions attributable to their more efficient axial cooling.

optimal for the ions with *m/z* values in the middle of the range. Because of the short cooling time, the spectrum exhibits poor signal-to-noise ratio and mass resolution. As expected, a significant signal enhancement can be achieved with longer ion–ion cooling time. Figure 2b shows the spectrum of the same mixture under the same experimental conditions, but after 10 s of ion–ion cooling (note that for different FTICR instruments this magnitude of spectrum enhancement can be achieved even for longer periods of time). Figure 2c shows the spectrum from the same mixture obtained under the same experimental conditions with 300 ms dynamic ion cooling. Figure 3 demonstrates how the time–domain transient changes when applying dynamic ion cooling. The transfer time was optimized for the 2+ charge state of bradykinin and provided good quality spectra (Figure 3a) for even short, 300 ms, ion–ion cooling periods (the ideal situation is when the ions of one *m/z* are captured in the center of the trap). For the same period of time, the use of the dynamic ion cooling method substantially improved both the signal intensity and the resolution (Figure 3b).

Of particular interest also was to find how the DIC method affects the charge state distribution in ESI mass spectra of proteins. Figure 4 shows a myoglobin spectrum obtained using 500 ms ion–ion cooling and 500 ms dynamic ion cooling. The transfer time between external accumulation and gated trapping was optimized for the ions in the middle of the charge state distribution. As a result of this time not being optimal for the charge states at the wings of the distribution, the signal quality is biased toward the center of the distribution, as is clearly seen in Figure 4a. The use of dynamic ion cooling restores the normal charge state distribution by enhancing the signals from ions with marginal *m/z* ratios.

A particularly interesting characteristic of the DIC method is its effect on mass accuracy. A mass self-consistency test was performed on spectra from a mixture of several peptides and insulin. The compari-

Table 1. Results for calibration of spectra under various ion cooling conditions

Peptide/cooling method		300 ms ion–ion cooling		10 s ion–ion cooling		300 ms dynamic ion cooling	
Name	Calculated mass	Measured mass	Error ppm	Measured mass	Error ppm	Measured mass	Error ppm
Renin Inh, 2+	513.2819713	513.3092210*	53.089*	513.2817908	−0.352	513.2819313	−0.078
Bradykinin, 2+	530.7879507	530.7867537	−2.255	530.7877985	−0.287	530.7879497	−0.002
Neurotensin, 3+	558.3104751	558.3109290	0.813	558.3103535	−0.218	558.314673	−0.014
Substance P, 2+	574.371317	674.3714802	0.242	674.3713648	0.071	674.3712838	−0.049
Fibrinopeptide, 2+	768.8498477	768.8495262	−0.418	768.8499805	0.173	768.8498988	0.066
Neurotensin, 2+	836.9620715	836.9641379	2.469	836.9623595	0.344	836.9622576	0.222
γ-Endorphin, 2+	929.9663581	929.9659304	−0.460	929.9668261	0.503	929.9661765	−0.195
Insulin, 5+	1147.528872	1147.528455	−0.364	114.528586	−0.249	1147.528891	0.016
Average error			**1.003**		**0.275**		**0.080**

* Not included in the calibration and average error calculation due to low peak quality

son of the average error obtained after calibration was made in each of the three cases: 300 ms ion–ion cooling, 10 s ion–ion cooling, and 300 ms dynamic ion cooling. In all three cases ions were detected at 0.5 V trapping potential, and the calibration equation included a quadratic term (taking into account magnetron frequency shift and a global space charge effect). Ions in these experiments were SWIFT [17] excited to an estimated half of the trap's radius. Time–domain transients were triangle apodized, one-zero-filled, and then fast Fourier transformed to obtain mass spectra in magnitude mode. The results are shown in Table 1. When DIC was employed the achieved average mass accuracy was as high as 80 ppb, and more than an order of magnitude better than in case of 300 ms ion cooling (even after removing poor split peaks from calibration). Mass accuracy was improved for every peak in the spectrum, even compared with 10 s ion–ion cooling.

Conclusion

Preliminary investigations of the dynamic ion cooling method, which is based upon the adiabatic invariant phenomenon, have demonstrated the potential for more rapid ion cooling in an FTICR trap. The period of time required for ion cooling without the use of a buffer gas can be as short as few hundred milliseconds for the ions in the *m/z* range of up to 1000. DIC also provides a high *m/z* bandwidth. We have also demonstrated that both resolution and peak intensity are significantly improved. Also, of particular importance is the significant increase in mass accuracy across a broad *m/z* range. For example, an average mass accuracy as high as 80 ppb has been achieved with 300 ms DIC. This technique may be combined with the ion axial dipolar, or parametric, weak (tickle) excitation, which should increase the efficiency of cooling for large ion populations (which have a weak periodicity of axial motion). We believe that DIC may be particularly useful in many new biological applications of FTICR, such as proteomics, and those involving extensive on-line separations when high duty cycle and high signal quality are particularly important.

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