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Communication

General implementation of the ERETIC™ method for pulsed field gradient probe heads

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ABSTRACT

A capacitive coupling between a secondary radiofrequency (rf) channel and the gradient coil of a standard commercially available high resolution NMR spectrometer and probe head is described and used to introduce a low level exponentially damped rf signal near the frequency of the primary rf channel to serve as an external concentration standard, in analogy to the so-called ERETIC™ method. The stability of this inexpensive and simple to implement method, here referred to as the Pulse Into the Gradient (PIG) approach, is superb over a 14-h period and both gradient tailored water suppression and one-dimensional imaging applications are provided. Since the low level signal is introduced via the pulsed field gradient coil, the coupling is identical to that for a free induction signal and thus the method proves to be immune (within 5%) to sample ionic strength effects up to the 2 M NaCl solutions explored here.

1. Introduction

It is well known that nuclear magnetic resonance (NMR) spectroscopy is quantitative, i.e. measured peak intensities directly correspond to nuclear spin concentrations [1]. This spectral intensity dependence combined with chemical shift and scalar *J* coupling resolution make NMR spectroscopy a powerful tool since the relative number of the different chemical environments and their geometric relationship in a molecule can be determined from the NMR spectrum [2]. The absolute nuclear spin concentration within a given molecular environment or at a given chemical shift cannot be determined directly from the spectrum without exhaustive spectrometer calibration or the inclusion of an internal standard with known concentration. Provided that a "friendly" internal standard can be found, i.e. a compound that does not react with, exists in the same phase as, and has a chemical shift different than the sample being studied or the analyte, the problem of absolute quantitation is solved. Here the ratio of the integrated peak intensity of the analyte to that for the standard multiplied by the standard concentration gives the analyte concentration. However, there are many situations where an internal standard cannot be used [3–5] and external referencing is required. One alternative approach that uses an electronic reference (ERETIC™) has become an increasingly popular option [6]. The ERETIC™ method uses a low voltage, exponentially damped, synthetic radiofrequency (rf) signal near the Larmor frequency of the analyte to effectively provide an external concentration standard with the main advantage being that the corresponding signal intensity is a faithful representation of electrical variations in the NMR detection circuitry. While all ERETIC™ implementations require either an additional spectrometer channel or a high frequency waveform generator clocked with the NMR instrument, two distinct modes of coupling the ERETIC™ signal into the NMR detection electronics have been presented in liquids and solids NMR as well as magnetic resonance imaging [6–13]. However, both approaches have their limitations. Historically, the first ERETIC™ applications coupled the low voltage calibration signal into the NMR detection electronics with a broadband untuned coil, an approach most efficiently and reproducibly accomplished by mounting the ERETIC™ antenna inside of the NMR probe head close to the rf pulsing/receiving coil [6,7,10]. Drawbacks to this approach are obvious. The NMR probe head must be customized to accommodate the broadcast antenna, an option that is not viable for most commercial NMR instrumentation, and the antenna also behaves as a receiver picking up high power rf pulses, an effect that at best decreases probe head efficiency and at worst damages the ERETIC™ signal generator. Both of these problems have been circumvented in more recent ERETIC™ applications, which use a directional coupler on the second channel of a double resonance NMR spectrometer and probe head to present the low voltage ERETIC™ signal to the sample and primary detection

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channel [8,9,11]. In this way the tuned LC circuit for one frequency is used to broadcast the ERETIC™ signal at another frequency. Although no NMR probe modifications are required, provided that a double resonance probe is used, in practice the method is extremely sensitive to the mutual coupling between rf channels, an effect that is controlled by the respective tuning and matching capacitors on both channels. In addition, as the rf isolation between channels improves, the performance of ERETIC™ drops. In spite of the concentration quantitation offered by the ERETIC™ method the implementation awkwardness described above limits its broad application, and thus alternative external referencing methods have been proposed [14].

This work describes a straightforward implementation of the ERETIC™ method that can be quickly integrated into standard spectrometers with the minimal addition of extra hardware. This scheme combines the positive aspects of both approaches described above to present the ERETIC™ signal to the detection electronics with only the addition of a simple capacitor. The method is completely general and can be implemented on any probe head equipped with a pulsed field gradient (PFG) coil, either in high-resolution or imaging setups, allowing the untuned PFG coil available in virtually all modern liquids or solids NMR probe heads to serve as an ERETIC™ antenna. An rf directional coupler is not needed as the ERETIC™ signal is capacitively coupled into the cable connecting the gradient amplifier to the gradient channel in the NMR probe head as shown in Fig. 1. The capacitive coupling of this method, hereafter referred to as the Pulse Into the Gradient (PIG) approach, has the benefit of electrically isolating the gradient channel from the low voltage ERETIC™ signal source, i.e. the long time scale μs–ms high current DC field gradient pulses do not feed back into the ERETIC™ channel, but the higher frequency ns–μs ERETIC™ signal couples into the gradient cable. The use of the gradient circuit included in the probe head offers several advantages in comparison to existing ERETIC™ signal coupling strategies. Since the PFG coil is not tuned, a flat frequency response is observed and the extremely small mismatch between rf and gradient coil positions along with their close proximity is enough to inductively couple the ERETIC™ signal into the detection electronics. The use of the untuned PFG coil as an ERETIC™ antenna guarantees the possibility of extending the method to very high field and thus Larmor frequencies of up to a few GHz. An additional attractive feature of the PIG approach is the concomitant increase in ERETIC™ signal stability offered by the properly shielded PFG coil and cables.

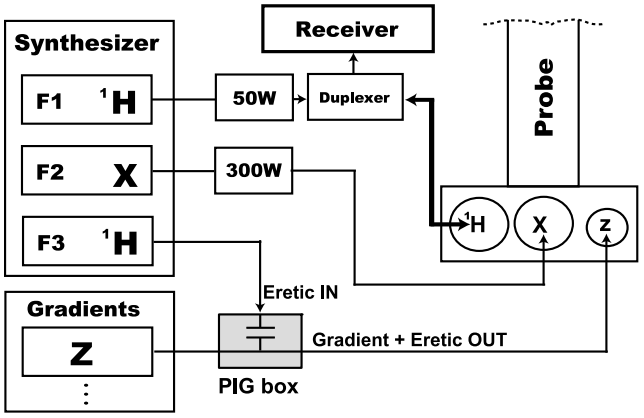

Fig. 1. Diagram showing three rf and one gradient NMR spectrometer outputs, rf amplifier, duplexer, receiver, and high resolution double resonance *z* gradient NMR probe head inputs. The PIG box (shown in gray) couples the ERETIC™ signal to the gradient coil through the use of two 1 kV 0.01 μF capacitors connected in parallel.

2. Experimental

All deuterated compounds were obtained from Cambridge Isotope Laboratories, while all other chemicals were obtained from Sigma–Aldrich and used without further purification. NMR spectra were collected using a 400 MHz Bruker Avance spectrometer equipped with two distinct commercial 5 mm *z* gradient Bruker probe heads. Specifically, the stability of the ERETIC™ signal was investigated using both a direct triple-resonance X/^{1}H/^{31}P TBO and an inverse ^{1}H/X BBI probe heads. All other experiments (WATERGATE [15], imaging, ionic strength monitoring) were conducted with the inverse probe head that offers the highest ^{1}H sensitivity. In addition, the frequency, amplitude, phase, and damping of the ERETIC™ reference signal synthesized in the third spectrometer channel (F3) were chosen to emulate a real NMR signal. In cases where a three-channel spectrometer is unavailable, the ERETIC™ signal can also be synthesized using the second rf channel. The synthesizer output is then directed to the untuned gradient coil through the PIG box (see Fig. 1), which is a simple home-made device based on a trivial electronic circuit made of two 1 kV 0.01 μF capacitors connected in parallel.

3. Results and discussion

As can be gathered from Fig. 1, the critical elements in this external signal referencing approach are the PIG box capacitors which are used to pass the high frequency low voltage ERETIC™ signal but block the high power DC field gradient pulses from reaching and damaging the ERETIC™ signal source, here the F3 spectrometer channel. The 18 mA current break through the capacitor when a 14.8 A 10 ms square DC gradient pulse is applied provides ca. 135 dB isolation between the gradient and F3 channel. The particular capacitance used here is not unique, higher or lower values can be chosen as long as enough isolation is provided to protect the F3 channel from the high current DC gradient pulses. The performance of the capacitive coupling of the ERETIC™ signal to the gradient channel was demonstrated in four different liquid state NMR applications.

The first measurement considered the long-term stability of the method by repeatedly recording a ^{1}H NMR spectrum with the added ERETIC™ signal over a 14-h period. The spectrum in Fig. 2a corresponds to the methyl group region in a 5 mM valine sample with 2 M added NaCl to mimic the high ionic strength common to biological samples. The ratio of the integral of the ERETIC™ signal (at 0 ppm and denoted by the asterisk in Fig. 2a) to the integral of the valine methyl groups peaks at ca. 1 ppm over this 14-h period is summarized in terms of a percent deviation from the average intensity ratio 1.007 in Fig. 2b. The 3.4×10^{-5} variance in the intensity ratio over the 14-h period embodied by the fluctuation about the average shown in Fig. 2b implies that the valine concentration can be specified as 5.000 ± 0.012 mM (at 95% confidence). This statistical measurement suggests that there is little variation in the ERETIC™ signal coupling efficiency over time and consequently little or no recalibration is needed when short-term data collection is performed.

The ability to perform external referencing in a standard high resolution liquid state NMR application that requires the use of pulsed magnetic field gradients was demonstrated in the second measurement by applying the WATERGATE water suppression pulse sequence [15] to a 10 mM phenylalanine sample dissolved in a 50% (v/v) H_2O/D_2O solution as shown in Fig. 3. The standard one rf pulse ^{1}H NMR spectrum without water suppression but with the ERETIC™ signal (extra peak at ca. −2.5 ppm) is shown in Fig. 3a. Application of the WATERGATE pulse sequence

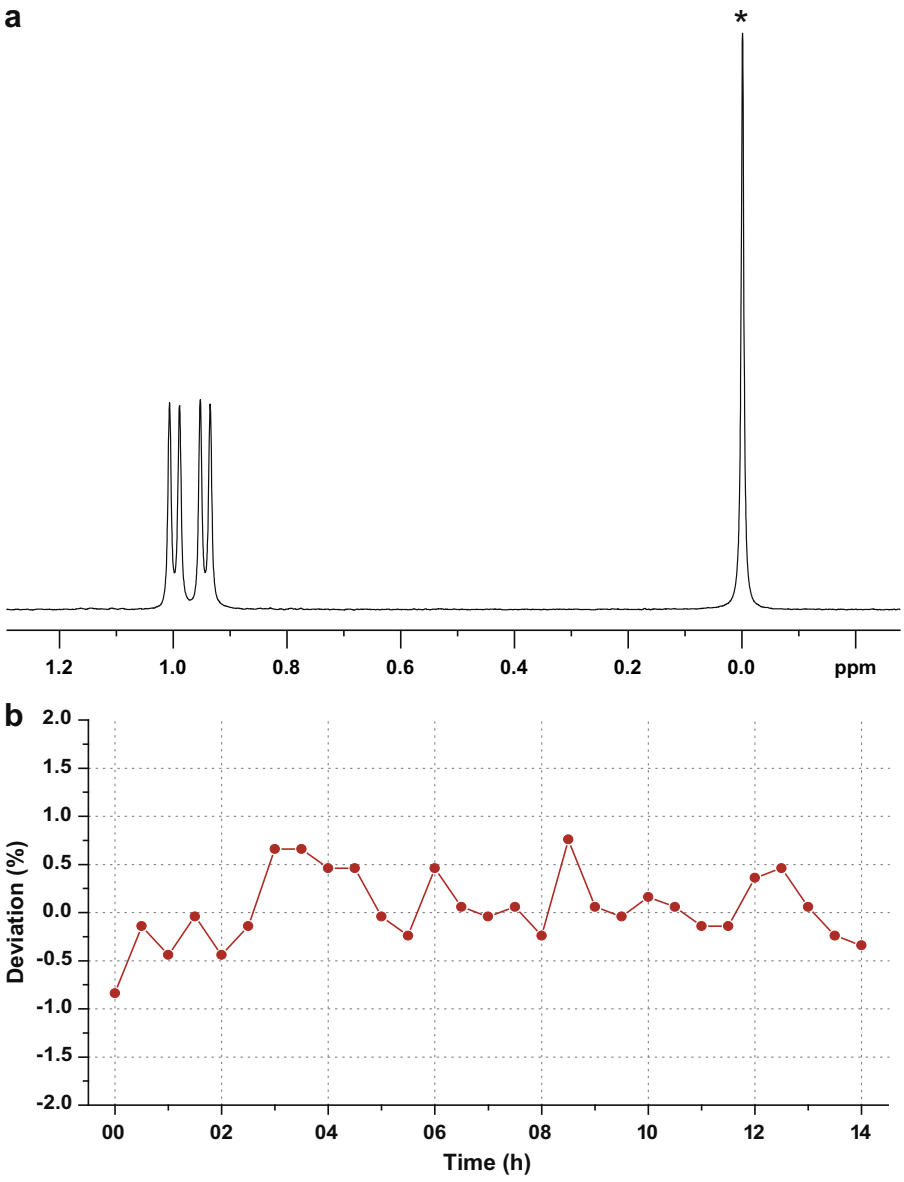

Fig. 2. (a) High resolution ^{1}H NMR spectrum of 5 mM valine in a 2 M NaCl/D_2O solution showing just the methyl group region. The ERETIC™ signal at 0 ppm is marked with an asterisk. (b) Plot showing the percent deviation from the average value of 1.007 of the ratio of the ERETIC™ signal integral to the valine methyl groups integral in the ^{1}H NMR ERETIC™ edited spectrum for a 14-h period. The standard deviation of these ratios over this period is 0.005.

significantly attenuates the intense water peak as shown by the spectra reported in Fig. 3b and c, which were recorded in the absence and in the presence of the ERETIC™ signal, respectively. Comparison of Fig. 3a with Fig. 3c and of 3b with 3c suggests that the fidelity of the ERETIC™ signal during the application of the field gradient pulses and the quality of the water suppression, respectively, are not affected by any interaction of the gradient with the ERETIC™ channel due to leakage through the isolation capacitor. This is a necessary requirement for routine implementation of the ERETIC™ method through the PIG approach on modern liquid state NMR instrumentation.

The results of the third set of experiments are shown in Fig. 4. Here two phantom 1 g/L $CuSO_4$ in 50% (v/v) H_2O/D_2O solutions were used to illustrate the feasibility of the PIG approach in a simple one-dimensional magnetic resonance imaging experiment. The first phantom sample is the 5 mm diameter continuous tube of liquid shown in Fig. 4a while the second contains a 2.2 mm wide spacer as shown in Fig. 4d. The one-dimensional images corresponding to these objects are shown without the added ERETIC™ signal in Fig. 4b and e, and with the ERETIC™ reference (denoted by an asterisk) in Fig. 4c and f. The rounded edges at ca. ±4 mm in all of these images reflect the tradeoff between gradient linearity and rf inhomogeneity as both phantom samples extend far beyond the ends of the rf coil.

Importantly, the similarity of Fig. 4b to Fig. 4c, and of Fig. 4e to Fig. 4f, indicates that the capacitive ERETIC™ signal coupling does not perturb the magnetic resonance image, an observation that implies that the rf ERETIC™ signal is at a sufficiently low amplitude to avoid any unexpected feedback problems with the PFG amplifiers.

The final experiment considered the reduction in NMR probe head efficiency commonly noticed in high ionic strength solutions. Here the increased ion concentration effectively increases the NMR circuit resistance, spoils the tank circuit quality factor Q, and thus decreases the sensitivity of the NMR experiment. The effect of tank circuit loading on the ERETIC™ signal coupling

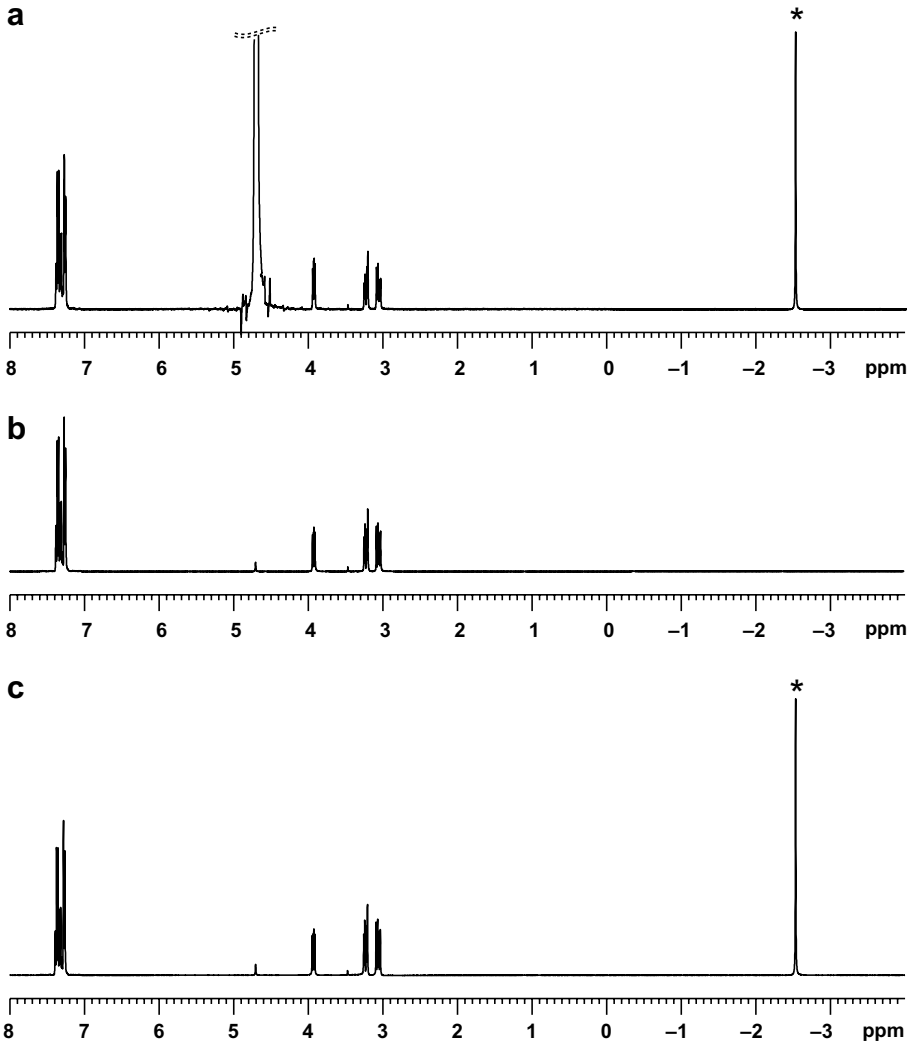

Fig. 3. (a) High resolution ^{1}H NMR spectrum of a 10 mM phenylalanine sample dissolved in a 50% (v/v) H_2O/D_2O solution doped with $CuSO_4$ at 1 g/L. Note that the water peak has been truncated. (b and c) WATERGATE spectra recorded on the same sample without (b) and with (c) the introduction of the ERETIC™ signal (denoted by an asterisk in a and c).

efficiency was explored by monitoring the high resolution ^{1}H NMR spectrum of six distinct 5 mM valine samples dissolved in D_2O at increasing NaCl concentration (from 0 to 2 M). Specifically, the integral of the methyl groups signals was monitored as a function of the NaCl concentration in three cases: (i) without 90 pulse recalibration, (ii) with 90 pulse recalibration, and (iii) with 90 pulse recalibration and ERETIC™ normalization (see Fig. 5). Note that 90 pulse recalibration means that the 90 pulse duration was changed. Moreover, in all cases, the tuning and matching settings of the probe head were not modified, in order to simulate the conditions typically encountered in automatic NMR analysis when automatic tuning capabilities are not available. In the first case, the signal integral strongly decreases while the NaCl concentration increases. For instance, for the 2 M NaCl sample, the integral of the valine methyl groups dropped down to 65% of its initial value.

While a slight improvement can be achieved by recalibrating the 90 pulse (75%), the best results were obtained when recalibrating the 90 pulse and normalizing the integral of the valine methyl groups to that of the ERETIC™ reference signal (95%). In other words, the use of the ERETIC™ signal allows for the compensation of the variation in electronics caused by the change in ionic strength for a wide range of NaCl concentrations. This compensation is mostly due to the concomitant decreases in the efficiency of both the NMR and ERETIC™ signal reception.

4. Conclusion

The primary goal of this work was to introduce a simple, practical, and inexpensive modification to a modern high resolution NMR spectrometer that permits external sample concentration referencing. The approach couples an ERETIC™ reference signal through a capacitor into the cable connecting the DC PFG amplifier to the PFG coil contained in most commercially available high resolution liquid and solid state NMR probe heads. The stability of the Pulse Into the Gradient (PIG) approach is superb and immune (within 5%) to ionic strength effects. Moreover, virtually all modern rf pulse sequences and experiments can be performed without any additional hardware modifications, as illustrated in both a water suppression and a one-dimensional magnetic resonance imaging application. The success of using the gradient coil as an ERETIC™ antenna recognized here suggests that the method can be generalized to NMR spectrometers without PFG capability. Here a capacitive coupling to the room temperature shims instead of the gradient coil

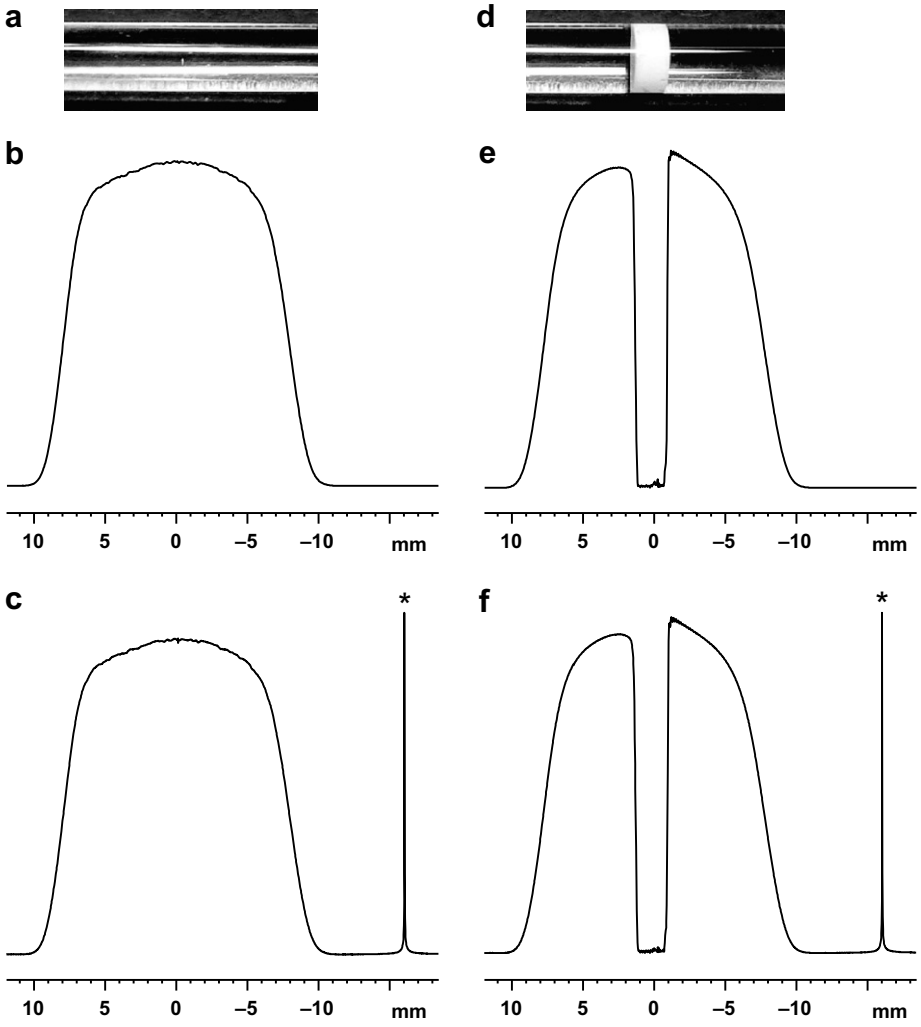

Fig. 4. Magnetic resonance images of two phantom samples shown in pictures (a and d). The standard one-dimensional images corresponding to these two samples are shown in (b and e), respectively, while (c and f) demonstrate the consequences of introducing the ERETIC™ signal denoted by an asterisk.

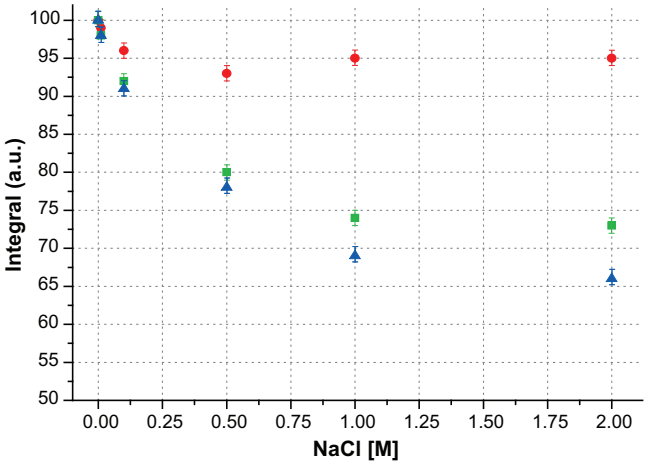

Fig. 5. Evolution of the integral of the signals due to the valine methyl groups as a function of NaCl concentration (0, 0.001, 0.01, 0.1, 0.5, 1, 2 M). Three cases are considered: (▲) without 90 pulse recalibration; (■) with 90 pulse recalibration; (●) with 90 pulse recalibration and ERETIC™ normalization.

could be used to accomplish the same external referencing goal realized in the experiments described above.

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