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ScienceDirect
Journal of Magnetic Resonance 187 (2007) 120–125
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Quantitative ^{2}H NMR spectroscopy with ^{1}H lock extender

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Received 22 November 2006; revised 20 March 2007
Available online 20 April 2007

Abstract

An inexpensive external unit that allows the use of the commercial high-resolution NMR spectrometer for ^{2}H observation with an ^{1}H lock system is described. The external unit does not require any tuning, is extremely easy to use, and could be a cheaper and more straightforward alternative to the more expensive ^{19}F lock configuration. An application for the quantitative determination of the natural isotopic ratio $^{2}H/^{1}H$ of ethanol and acetic acid is reported.
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Keywords: Quantitative deuterium NMR; Proton lock; Ethanol; Acetic acid; SNIF-NMR

1. Introduction

^{2}H NMR can be usefully employed in food science. In 1981, it was shown that deuterium atoms at natural abundance level exhibit large deviations compared to a statistical distribution (0.01%) in the different sites of a molecule [1], and a new method based on quantitative deuterium NMR for the determination of site-specific natural isotope fractionation (SNIF-NMR) [2,3] was developed. For a given natural substance, SNIF-NMR gives information about the chemical pathway of biosynthesis, and in some cases also about the geographical origin of the sample [4]. Deuterium analysis by NMR has thus become a powerful tool for food authentication, and a procedure for detecting and measuring the enrichment of wines has been defined on this basis, becoming, in 1990, the first officially adopted stable isotope method in the EU for wine analysis. The results collected provide a means for identifying the provenance of European wines [5–8].

Besides ethanol in wines, SNIF-NMR was applied to other natural food components, such as acetic acid in vinegar [9,10], and citric acid in fruit juices [11,12].

The application of the SNIF-NMR method to deuterium is limited by the fact that normal NMR instruments use deuterium as a lock, so there are two choices when observing ^{2}H NMR signal, i.e. running the instrument unlocked, or using the ^{19}F lock. The NMR analysis without lock presents the problem of instrument drift, which is particularly significant when analysis must cover a long period of time (as is the case with natural abundance deuterium determination). The ^{19}F lock configuration has some evident drawbacks, as a special channel (receiver, transmitter and probe), usually very expensive, must be installed and tuned to ^{19}F, and an appropriate fluorine containing solution must be added in the probe coil, in some cases physically separated from the sample under study (i.e. in a capillary inside the sample tube). In the latter case some difficulties in obtaining good magnetic field homogeneity must be taken into account.

On principle, it is also possible to lock to ^{1}H nuclei, as had been done in the old NMR instruments or in special applications [13], obtaining a very sharp NMR line at high frequency, which is very suitable for locking. Unfortunately, this facility is not commonly available in modern commercial spectrometers, thus limiting the application of quantitative ^{2}H NMR, which is very interesting for food science.

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doi:10.1016/j.jmr.2007.04.008

Here we describe an inexpensive external unit, realized by means of a recycled item and a simple passive converter, which allows the observation of deuterium using ^{1}H as a lock, and this way avoids any modification to the commercial spectrometer (the standard high-resolution multinuclear probe is used) and any special preparation of the sample (locking is achieved on ^{1}H nuclei of every substance in the sample that gives a suitable signal).

Our external unit offers good overall performance in unmodified samples and has been employed successfully in ^{2}H measurements of ethanol from wines and acetic acid from vinegars.

2. Experiment

NMR analyses of deuterium at natural abundance level were carried out on standard solutions of ethanol and acetic acid (kindly supplied by the Istituto Agrario S. Michele all' Adige, Italy); TMU (*N*,*N*-tetramethylurea with known D/H isotopic content, supplied by the Institute for Reference Materials and Measurements, Belgium) was used as internal standard.

Table 1
Acquisition and processing parameters

Parameter	Value
Time domain points	16384
Spectral width	4 kHz
Sample spin	On
Spectrum data point	32768
Line broadening	0 Hz
Temperature	25 °C
Recycle delay	4.8 s
Number of scans	1000
Acquisition time	2.02 s
Observed pulse (90°)	12 μs

Exactly weighed TMU (1.1 g) were added to 3 g (exactly weighed) of ethanol or acetic acid standard, and placed in a 10 mm NMR sample tube for the deuterium analysis.

Deuterium spectra were recorded at 61.42 MHz on a Bruker AMX 400 spectrometer, with a 10 mm probe and a wide bore magnet, under the instrumental conditions reported in Table 1. Spectra were acquired both unlocked with the WALTZ16 proton decoupling, and ^{1}H-locked, by means of the external unit, without proton decoupling.

Data from 1000 pulses were recorded for each experiment, in order to achieve a satisfactory signal to noise ratio and 10 replicates were performed for each sample. The spectra were carefully baseline corrected and manually integrated: no lineshape simulation was performed; the manual integration was carried out by cutting the peak tails at 10 Δ points, where Δ is the half height–half linewidth.

The isotope ratio of the methyl group of acetic acid or ethanol (expressed as part per million of deuterium compared to proton, ppm) was calculated according to Eq. (1):

$$\mathrm{D/H} = \frac{12}{3} \times \frac{M_x}{M_{tmu}} \times \frac{m_{tmu}}{m_x} \times \frac{A_x}{A_{tmu}} \times (\mathrm{D/H})_{tmu} \quad (1)$$

where M_x, molecular weight of acetic acid or ethanol; M_{tmu}, molecular weight of tetramethylurea; m_x, weighed mass of acetic acid or ethanol; m_{tmu}, weighed mass of TMU; A_x, NMR signal area of $-CH_2D$ of acetic acid or ethanol; A_{tmu}, NMR signal area of $-CH_2D$ of tetramethylurea; $(D/H)_{tmu}$, certified deuterium content of tetramethylurea provided by supplier.

3. Results and discussion

3.1. Configuration of the external unit

The general outline of the external unit is shown in Fig. 1. The unit implements a simple heterodyne scheme for both the receiving and transmitting sections, plus a

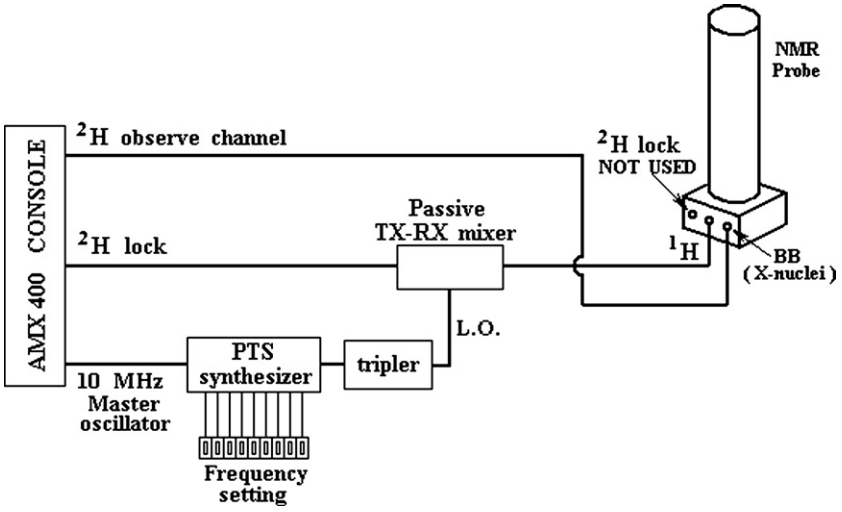

Fig. 1. Block diagram of the external unit. Because of the electronic configuration, the ^{1}H lock NMR signal appears on the commercial spectrometer (Bruker AMX 400, in our case) as a usual deuterium lock signal.

fixed frequency reference (local oscillator, L.O.) supplied by an external synthesizer.

A local oscillator frequency of about 338 MHz must be obtained from the synthesizer at about +10 dBm level

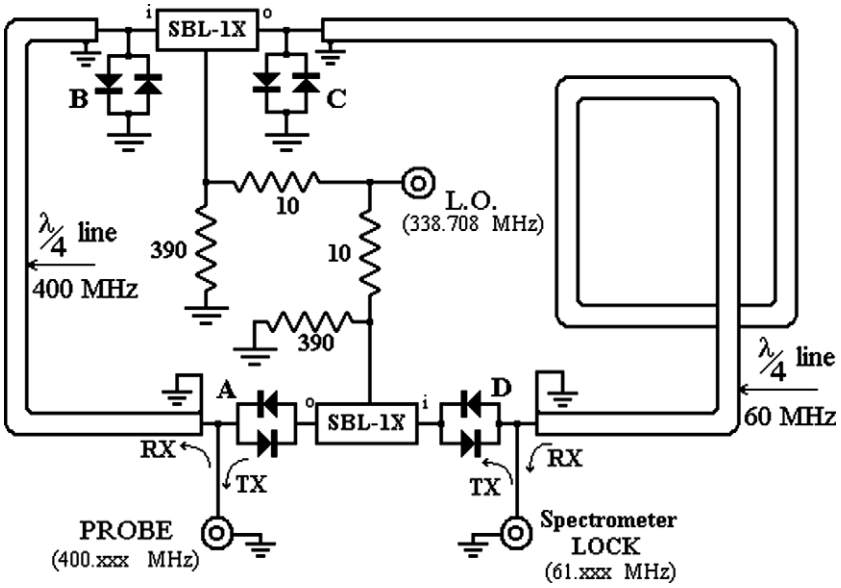

Fig. 2. TX-RX passive mixer. All diodes are HP 5082-2800. Both mixers are Mini Circuits SBL-1X (double balanced mixer, +7.5 dBm L.O. level). Resistor values are given in ohm. Both quarter-wavelength lines are made of 50 ohm coaxial cable standard flexible type RG-142 (dual silver shields, extruded PTFE dielectric, velocity factor = 0.695); the 400 MHz quarter-wavelength line is 13 cm long, the 60 MHz quarter-wavelength line is 86 cm long (few millimeters approx).

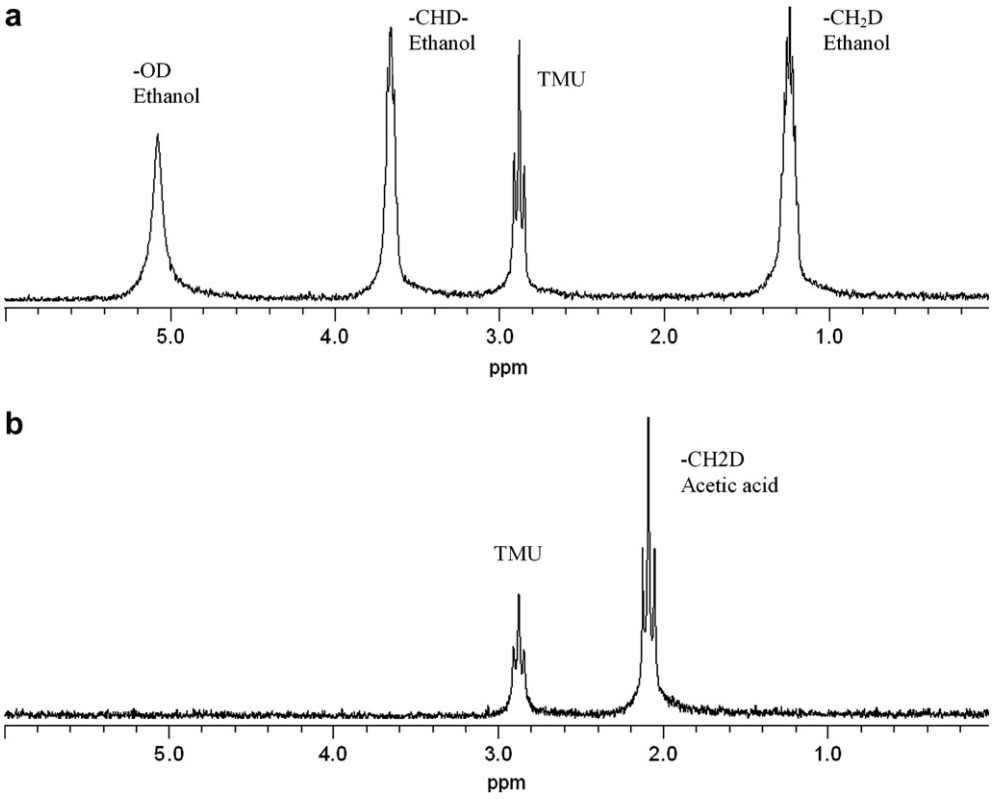

Fig. 3. ^{2}H NMR spectra of (a) standard ethanol and (b) standard acetic acid, registered with the 1H lock obtained by means of the external unit on a Brucker AMX-400 instrument.

(10 MHz 'external reference' is picked up from the spectrometer master oscillator).

We had a PTS synthesizer, part of a dismantled old NMR spectrometer, but, as its maximum frequency is 160 MHz, it has been necessary to add a standard transistorized frequency tripler.

Frequency setting of our synthesizer was achieved by means of 8 BCD multiswitches; in our case the frequency was 113,333,000 Hz, so that, once multiplied by a frequency tripler, the local oscillator frequency of 338,708,000 Hz was obtained.

This looks like a rather conventional approach compared to the design of the most modern spectrometers in which a high frequency signal is performed directly by a digital synthesizer (DDS).

We chose this way because of the simple construction and inexpensive items.

A remarkable characteristic of the RX-TX mixer (Fig. 2) is that is totally passive, i.e. it does not need any power supply.

The lock pulses coming from the console reach the passive mixer through a TX-RX switch. Diodes are employed on the transmitter and receiver arms for passive switching, i.e. switching is provided by the RF excitation itself [14].

The r.f. lock pulses are actually not very strong ($1 \div 2$ V, adjustable by the lock power command on the console), so Schottky barrier diodes (type HP 5082-2800) were used in order to take advantage of their low barrier voltage ($0.2 \div 0.3$ V).

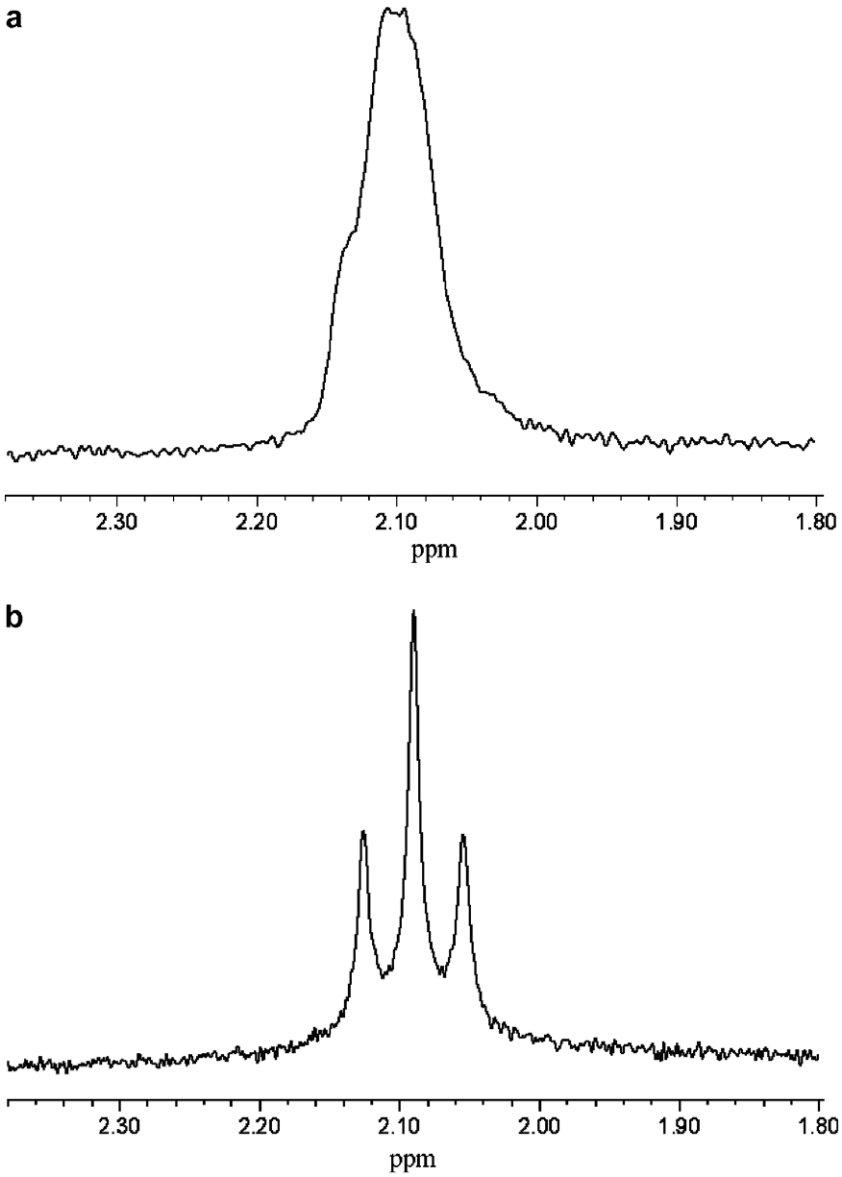

Fig. 4. Comparison between signal shapes of acetic acid in the deuterium NMR spectrum registered (a) without lock and (b) with proton lock by means of the external unit.

During the application of the radio-frequency lock pulses, all sets of crossed diodes conduct heavily and present low impedance. The effective short circuits at B and C (Fig. 2) appear as open circuits at the probe and lock connectors due to the impedance transformation properties of the two quarter-wavelength lines.

Any quarter-wave line acts as an impedance transformer according to the equation: $Z_{in} = Z_o^2/Z_{out}$ where Z_o is the characteristic impedance of the transmission line. The one quarter-wavelength line changes a short circuit into an open circuit.

Thus the r.f. lock pulses pass through the crossed diodes A and D and reach the probe with no appreciable attenuation except that due to the frequency conversion.

Following the lock pulses the diodes stop conducting; A, B, C and D crossed diodes present very high impedance. They act like open circuits (voltage across diodes is too small to turn them on), allowing the small ^{1}H NMR lock signal from the probe to reach the mixer; here it is converted into a 60 MHz lock signal and then conveyed to the lock connector through common 50 Ω lines.

In the case of a very low level of the lock pulses from the spectrometer, the input set of crossed diodes to the TX mixer (diodes D) may be removed to avoid the doubling of the threshold level during transmission. As a consequence, some loss of signal and added noise in the receiving path are expected. However, due to the very high lock signal coming from the protons of the sample, this did not appear to be a problem.

3.2. Application of the 1H lock system to the ^{2}H NMR quantitative analysis of natural abundance deuterium in ethanol and acetic acid

The ^{2}H NMR spectra of ethanol and acetic acid standard solutions, registered with our 1H lock system, are reported in Fig. 3. The spectra, processed with no line broadening correction, show the multiplicity due to the coupling with proton. The spectral resolution was about 0.5 Hz at half height, while for the unlocked spectra it was higher than 4 Hz. This is related to the magnetic drift which can occur in long-time analysis, as in the measurement of deuterium at natural abundance level, and can produce a significant enlargement of the signals. The different signal shapes, obtained without lock (Fig. 4a) and with ^{1}H lock (Fig. 4b) for acetic acid signal, are clearly shown in Fig. 4.

The better resolution and the more regular lineshape obtained for the samples registered with ^{1}H lock are directly related to the improved precision of the measurement, because we have found that the wide tails of the signals in the unlocked spectra make the integration difficult and lower the precision of the measurement. The comparison between the D/H values obtained for ten replicates of the analysis of standard ethanol and acetic acid solutions is reported in Table 2, showing that the measurements obtained with ^{1}H lock by the external unit are more precise than those obtained without lock. Moreover, comparing the D/H values of ethanol obtained with the certified D/H value, the ^{1}H-locked measurement can be claimed to be more reliable too. It is important to point out that our results are accurate even if the line shape was not optimal due to the use of 10 mm NMR tubes and a wide bore magnet, which causes great difficulty in obtaining good homogeneity. We expect better results with a more suitable NMR spectrometer.

Table 2
Comparison between the D/H values (expressed as ppm, part per million of deuterium compared to proton) for ethanol and acetic acid obtained without lock and with the proton lock by the external unit (average data of 10 experiments)

	D/H[a] CH_2D-ethanol without lock	D/H[a] CH_2D-ethanol with proton lock	D/H CH_2D-acetic acid without lock	D/H CH_2D-acetic acid with proton lock
Average	101.0	103.4	127.3	132.5
Standard deviation	3.0	0.9	5.0	1.5
Standard deviation (%)	2.9	0.9	3.9	1.1

[a] Certified D/H value = 102.37–103.55 ppm.

The only drawback of our system is that, at present, it does not allow the simultaneous 1H decoupling. The 1H undecoupled experiment causes a loss in sensitivity, due to the signal multiplicity. However, in the case of deuterium determination at natural abundance level this fact is not a great problem, because this particular analysis can be applied only to small and simple molecules [15]. We found that the advantages given by 1H lock overcome the problem of 1H undecoupling in terms of accuracy and precision of the measurement (Table 2).

4. Conclusions

The inexpensive external unit presented in this paper to obtain the ^{1}H lock was used with a Bruker AMX-400 NMR spectrometer to successfully determine the site-specific ^{2}H contents of ethanol and acetic acid.

The resolution, the precision and the accuracy obtained are compatible with those of European standards [16], so our modified NMR instrument can be utilized to determine the D/H ratio of ethanol and acetic acid on real samples of wine and vinegar.

Although our experience is limited to the AMX console working at 400 MHz (^{1}H nuclei), the same approach can be extended to any other NMR consoles, provided that the right local oscillator reference signal is fed to the mixers and the lengths of the quarter-wavelength lines are changed accordingly.

Finally, on the basis of these encouraging results, we are now working to adapt our external unit to simultaneous

1H lock and 1H decoupling, because this will permit a wider range of application of deuterium NMR analysis.

Acknowledgment

We are grateful to Dr. Federica Camin (Istituto Agrario San Michele all' Adige, Italy) for supplying the certified standard of ethanol and tetramethylurea.

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