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Receiver gain function: the actual NMR receiver gain

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Abstract

The observed NMR signal size depends on the receiver gain parameter. We propose a receiver gain function to characterize how much the raw FID is amplified by the receiver as a function of the receiver gain setting. Although the receiver is linear for a fixed gain setting, the actual gain of the receiver may differ from what the gain setting suggests. Nevertheless, for a given receiver, we demonstrate that the receiver gain function can be calibrated. Such a calibration enables accurate comparison of separately acquired NMR signals in quantitative analysis, which frequently requires different receiver gain settings to avoid receiver saturation or achieve optimum sensitivity. The application of receiver gain function, along with the definition of receiving efficiency, allows easy concentration determination by a single internal or external concentration reference.

Keywords

NMR; receiver gain; receiver gain function; quantitative analysis

Introduction

The NMR receiver gain is a parameter that is often chosen to maximize the signal to noise ratio. For optimum sensitivity, a dilute analyte needs to be observed with a high NMR receiver gain and the strong, interfering solvent signal has to be suppressed.^[1] On the other hand, a strong solvent or solute signal would be best observed with a low receiver gain so as not to saturate the receiver. This situation may occur, for example, in an accurate concentration determination method that we recently developed using solvent signal as reference.^[2] In such a method, the solvent proton concentration can be one million times higher than that of the analyte. To compare NMR signals acquired with different receiver gains, one would desire to know how much the NMR signal is actually amplified by the receiver.

More generally, the observed signal size for a modern high-resolution NMR can be expressed as

$$A = A_0 \times c \times V \times \mathfrak{R} \times \sin(\theta) \times I(\theta) \times g(\text{RG}) \quad (1)$$

where the terms describing an instrument constant A_0 , the sample concentration c , sample volume V , receiving efficiency $\mathfrak{R}$, pulse angle θ and the RF inhomogeneity factor $I(\theta)$, and receiver gain function $g(\text{RG})$, have been described previously.^[3] The receiver gain function characterizes the actual FID amplification achieved by the receiver, with a larger RG indicating higher receiver amplification.

For quantitation purposes, Eqn (1) clearly demonstrates that the receiver gain function is as important as the excitation angle or receiving efficiency, which were the focal points of our two previous studies.^[2,3] As long as the FID is not saturating the receiver, it is highly preferred that the actual observed NMR signal or the receiver gain function is linearly proportional to the receiver gain. In our experience, this situation has not always been the case. To overcome potential inconsistent receiver behavior in the amplification of the raw FID signal, a referencing method termed ERETIC was proposed as an artificial quantitation standard.^[4] However, the implementation of this approach is not always straightforward. In particular, ERETIC is not applicable if a constant sample load or interaction between the sample and coils cannot be maintained. In some cases, a solvent satellite or residual solvent signal can be used to quantitate natural products.^[5,6] Nevertheless, these methods have a number of limitations including the presence of the reference signal and knowledge of its accurate concentration.

Alternatively, the parameter of receiver gain can be kept as a fixed constant for separate acquisitions when the solvent is used as the concentration reference.^[2] Apparently, this method lacks the flexibility to deal with NMR signal of different amplitudes in quantitative analysis: for a strong signal, the gain has to be low to avoid the receiver saturation; for a weak signal, the gain has to be set high for optimum sensitivity.^[1] Therefore, we need the freedom to adjust the receiver gain for various analytes and solvents.

We have consistently observed that certain receiver gain settings on a given spectrometer result in over- or under-amplification than the receiver gain value suggests, regardless of the raw signal size. We believe it is possible and helpful to determine the relationship between the actual observed gain and the receiver gain parameter or setting. This process is very similar to transmitter linearization that is often implemented by major NMR spectrometer vendors. Nevertheless, the receiver gain function differs in that it appears to be a discrete function of the receiver gain. Instead of attempting to find a smooth correlation between actual gain and the receiver gain parameter, we would suggest calibrating the receiver gain function for a number of discrete gains. As such, NMR signals (FIDs or Fourier transformed spectra), acquired with different receiver gain settings, can be conveniently compared with each other in quantitative analysis.

Experimental

The 1D proton spectra were acquired using either a 2-Hz doped 20% H_2O or 0.1% H_2O in D_2O sample on the following spectrometers: a Bruker AVANCE 800 MHz with an RT inverse probe, an AVANCE 500 MHz with a broad band probe and another with a proton cryoprobe, an ARX 300 MHz with a quadruple-nucleus probe, a Varian INOVA 600 MHz with a proton inverse probe and an INOVA 300 MHz with a quadruple-nucleus probe.

For data acquisition on a Bruker spectrometer, the receiver gain was set to 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 or 1024. For a Varian spectrometer, the receiver gain was set to 0, 4, 8, 12, 16, 20, 24, 26, 28, 32, 36 or 40 dB. If a certain gain setting could not be achieved, the nearest possible number was accepted. The gains in dB (for Varian spectrometers) were converted to linear numbers (*vide infra*) in making Fig. 1(b).

The transmitter carrier frequency was generally placed on the water resonance and the sweep width was 10 ppm with a total of 16 k complex time domain points. Small excitation angles coupled with long relaxation delay ensured that interesting proton magnetization effectively reached steady states prior to any excitation pulse, and the receiver was not saturated even with the highest receiver gain in the current study (1024 for Bruker or 40 dB for Varian spectrometers). The pulse lengths were generally 4 μ s or slightly longer and the pulse angles were adjusted through the use of transmitter attenuation.

For data processing, a mild exponential window function (equivalent to 0.5 Hz line broadening) was applied before Fourier transformation. All spectra were manually phased and first-order baseline corrections were applied. The water signal was generally integrated over a range equal to 50 times the observed full peak width at half maximum.

Results

In most applications, only the relative receiver gain function is needed. The receiver gain function with the lowest gain setting is defined as 1. For a Bruker spectrometer, the lowest integer receiver gain setting is 1; for a Varian spectrometer, the gain is 0 dB and should be converted to $RG = 1$ for ease of data presentation. As such, $g(RG = 1) = 1$. In all further discussions, the receiver gain function at any other receiver gain setting can be calculated as:

$$g(RG) = A(RG) / A(RG=1) \quad (2)$$

where A is the observed NMR signal amplitude or peak integration normalized by the corresponding number of scans. The $g(RG)/RG$ ratio then readily characterizes how the receiver gain function deviates from the ideal value of 1.

Figure 1 shows scatter plots of $g(RG)/RG$ for a number of typical receiver gain settings from two representative NMR spectrometers (Bruker AVANCE 800 and Varian INOVA 600). In each plot, the value of $g(RG)/RG$ is an average of three measurements. The standard deviation is generally about 1% or less and hence not plotted as error bars, thanks to the excellent sensitivity and instrument stability achieved by these two high-field spectrometers. Similar plots for four other spectrometers can be found in the supporting information.

For a given spectrometer, the normalized $g(RG)$ value is roughly linearly proportional to the receiver gain at most receiver gains (less than 1000), with a standard deviation of approximately 5%. However, certain receiver gains may show large deviations from this linear relationship, depending on the spectrometer. In Fig. 1(a), as an example, the actual gain for the AVANCE 800 is less than $0.93 \times RG$ when the gain is 128. On the other hand, it is more than $1.07 \times RG$ for the gain parameter of 912. If the NMR signals with these two receiver gain settings need to be compared, the simple assumption of the receiver gain function's linear dependence on gain can easily contribute to a 15% error!

Therefore, such a linear assumption with a slope of 1 cannot be taken for granted in quantitative analysis. Instead, we strongly propose that only a smaller set of receiver gains be used in most applications, because the receiver gain function for those corresponding gains can be easily calibrated. Of course, this set of receiver gains should contain both low gains to detect strong NMR signals and high gains to achieve optimum sensitivity for dilute species.

To illustrate the utility of a standardized receiver gain function further, we used the solvent signal as an internal concentration reference to determine the concentration of sodium acetate (prepared as 7.20 mM) in a $D_2O : H_2O = 1.8 : 98.2$ solution on the AVANCE 800

spectrometer. Due to the dramatic concentration difference between solvent water and the analyte, it is difficult to observe the analyte with optimum sensitivity without saturating the receiver. As such, the water signal was observed with a receiver gain of 4, after a 1.42° pulse excitation. Separately, the analyte's methyl signal was observed with a receiver gain of 64 and an excitation pulse angle of 45° after a long relaxation delay which includes brief and weak water pre-saturation. If the receiver gain function in Eqn (1) is simply treated as linearly proportional to the receiver gain, then the sodium acetate concentration is calculated as 6.50 mM, which is about 15% lower than the expected 7.20 mM. However, with the calibration of the receiver gain function shown in Fig. 1(a), the sodium acetate concentration can be easily corrected to 7.22 mM.

Discussion

The NMR receiver typically consists of a cascade of high performance analogue amplifiers, which are turned on and off through receiver gain controls, along with one or more frequency mixers. Although individual amplifiers generally have very similar performance, the actual gain for a particular amplifier is difficult to predefine. Hence it is not surprising to see that the receiver gain function is only approximately proportional to the receiver gain. Nevertheless, it is not difficult at all to measure the actual gain achieved for each receiver gain setting after the receiver is built. Thereafter, we can define the receiver gain function and conduct its calibration. We use the term 'receiver gain function' to differentiate it from the parameter 'receiver gain' (or 'gain') and to stress its dependence on the parameter of receiver gain. Because each individual amplifier is different and independent of one another, the correlation of the receiver gain function values at two adjacent receiver gains may not be strong. In our opinion, attempts to correlate the receiver gain function for all possible receiver gain values would not be cost-effective. For this reason, we decided to focus on the calibration of receiver gain function for a limited number of gain settings rather than analyzing the internal details for why the gain function is not strictly linearly dependent on the receiver gain.

To analyze the impact of the input FID signal size to the receiver gain function, we can re-examine Eqn (1). Under a fixed set of experiment conditions, the observed NMR signal is proportional to $\sin(\theta) I(\theta) g(RG)$. For small excitation angles and modern NMR probes of high RF homogeneity, $I(\theta)$ can be treated as a constant. The observed NMR signal normalized by $\sin(\theta)$ should be a constant, if the receiver gain function $g(RG)$ does not depend on θ or the size of the raw FID signal input to the receiver. Figure 2a demonstrates that this is indeed the case for a representative receiver gain of 128 on the AVANCE 800.

Alternatively, we can manipulate the FID signal size by changing the transmitter power. For an example, on a Bruker ARX300 spectrometer, we increased the FID signal size by approximately eightfold and re-measured $g(RG)/RG$. In Fig. 2(b), the solid squares represent the measured $g(RG)/RG$ values for the FID obtained with a small excitation angle, and error bars represent standard deviations (they are generally larger than those in Fig. 1, due to lower spectrometer sensitivity). The solid circles are the $g(RG)/RG$ values calculated using eightfold higher transmitter power. For ease of presentation, the circles have been shifted slightly to the right for the same receiver gain. If $g(RG)/RG$ depends on the input FID signal size, then one would expect an upward displacement of the solid circles compared with the squares that have eightfold larger receiver gains. However, such a shift is not observed. Instead, the solid squares and circles overlap with each other within the experimental error, suggesting that $g(RG)/RG$ is indifferent to the size of the observed signal.

Similar behavior was consistently observed for all other spectrometers in the study (data not shown) as long as all other parameters remained the same. For most quantitative analyses

with errors on the order of 2%, it is sufficient to conclude that the receiver gain function is solely dependent on the receiver gain. In fact, either the input signal amplitude or the receiver gain parameter has to be adjusted so that the receiver linearly amplifies all data points within the same FID. If a linear receiver cannot be achieved, spectral artifacts such as images or rolling baselines will arise.^[7] However, those artifacts may not be clearly visible if the deviation from linear behavior is very small for the receiver. We will demonstrate elsewhere a method to detect subtle line-shape changes for very small receiver gain compressions.

The difference between the NMR receiving efficiency and receiver gain function should be stressed here: the receiving efficiency describes how efficient a unit NMR signal from the RF coil can be passed to the receiver as the input.^[3] Fundamentally, the receiving efficiency depends on the complete RF circuit that includes the NMR sample and the probe-matching network. Hence, the receiving efficiency is not related to the receiver gain setting or any signal conditioning thereafter. On the other hand, the receiving gain function depends on the electronic properties of the receiver and is indifferent to upstream events.

In combination with the comprehensive analysis of the receiving efficiency,^[3] the current characterization of the receiver gain function allows the complete correlation between the observed NMR signal and transverse magnetization in the sample. With a single external or solvent reference, an analyte's concentration can be accurately determined. While it is not the intention of the authors to shed detailed insights into the mechanism as to why the receiver gain function deviates from a linear dependence on the receiver gain parameter, it is sufficient to define the receiver gain function for all practical purposes. Although we recognize that the receiver performance is influenced by the ambient temperature, we have not observed any significant change of receiver gain function for a given spectrometer during the course of our study. Whenever needed, the receiver gain function can be simply calibrated as a discrete function of the receiver gain by a standard sample. Demonstration of its minimal dependence on the input FID signal size not only supports that the definition of the receiver gain function is meaningful but also is consistent with our previous results that almost any known NMR signal, especially the strong solvent signal is an excellent practical and cost-effective concentration standard.^[2]

Conclusions

We propose the receiver gain function to characterize the actual gain that an NMR receiver achieves quantitatively. We have demonstrated that the NMR receiver gain function can be calibrated as a discrete function of the receiver gain, regardless of input FID signal size. The simple assumption of a linear receiver gain function may cause significant errors in quantitative analysis unless high performance internal standards are used, which in some cases may not be possible. The effort or cost of the receiver gain function calibration is minimal as it is a purely receiver-specific property, which is independent of upstream events such as NMR sample or pulse excitation. The benefit is significant, because accurate comparison of FIDs or spectra acquired with different receiver gains can be easily achieved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

After normalization by the receiver gain (RG), the receiver gain function can be readily calibrated against a set of discrete receiver gain settings for a modern NMR spectrometer. To save space, only two representative calibrations are shown here for (a) a Bruker AVANCE 800 and (b) a Varian INOVA600. Although the correlation is highly reproducible for a given spectrometer, no simple linear relationship is found between the receiver gain function and receiver gain.

Figure 2.

The receiver gain function is minimally dependent on the input raw FID size to the receiver for (a) a Bruker AVANCE 800 and (b) a Bruker ARX 300. In (a), at a given receiver gain (128), the observed NMR signal is a constant when normalized by sine of the excitation angle θ (in degrees). In (b), the normalized receiver gain function ($g(\text{RG})/\text{RG}$) remains essentially the same when the raw FID is eightfold larger (filled circles) compared with the average of a small FID input (filled squares). Error bars represent the standard deviations, which are mainly contributed by the limited sensitivity for this spectrometer.