



## A method for improved quantification of $^1\text{H}$ NMR signals under low-resolution conditions for solids

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### ABSTRACT

Accurate determination of  $^1\text{H}$  NMR signal intensities is useful for quantitative analysis of the hydrogen content and also to determine the relative peak intensity ratios in different application scenarios. To this end we have investigated the reliability and sources of intensity errors in  $^1\text{H}$  solid-state MAS NMR. If sufficient resolution can be achieved by very high spinning speeds and high magnetic fields, quantification is straight forward. However, for poorly resolved spectra we show that small phase errors add a considerable amount of uncertainty. An analytical expression for the phase induced intensity-errors allowed us to suggest a robust and reliable recipe which is based on a combination of the spin-echo experiment, an extrapolation technique and a deconvolution algorithm which includes fitting of the signal phase. It significantly reduces errors caused by phase distortions, homonuclear dipolar dephasing, the receiver dead time delay and baseline rolling. The method was validated experimentally on samples with strong homonuclear dipolar interactions.

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### 1. Introduction

One of the attractive features of NMR is that signals are proportional to the number of detected spins, which allows to perform quantitative analysis by NMR (qNMR). Unlike ordinary chemical analysis by other chemical or physical methods NMR not only allows to determine the total amount of an NMR active isotope but also its amount in different chemical environments.

Since qNMR is well established in liquid-state NMR [1,2] here we focus on quantification of solid-state NMR spectra. Under typical conditions the sensitivity of the NMR experiment is rather limited which does not make NMR a likely candidate for trace analysis where the analyte is below 1% in concentration. Applications of solid-state qNMR are wide spread too, covering different areas as, for example pharmaceutical formulation [3,4], cement base materials [5,6], drugs [7–9], coals [10] or amorphous materials [11] and different nuclei like  $^{13}\text{C}$  [3,9,12–14],  $^{29}\text{Si}$  [13],  $^{119}\text{Sn}$  [13] and  $^{23}\text{Na}$  [15]. Interestingly methods based on  $^{13}\text{C}$ -CP-MAS NMR have proved to be quantifiable for organic matter [13].

A particularly tempting target/analyte for quantification are hydrogen atoms. In solids the hydrogen content is usually quantified by combustion analysis [16] which gives reliable results in many cases. However,  $^1\text{H}$  solid-state NMR is a very sensitive probe and the resolution has improved significantly even in single-pulse

excitation (SPE) experiments with the advent of very fast magic-angle-spinning probes [17]. Moreover,  $^1\text{H}$  qNMR allows to distinguish between chemically relevant hydrogen and hydrogen coming from impurities (e.g. laboratory grease) and works even in cases where simple combustion analysis fails because of the stability of the samples as in case of ceramics or some inorganic solids.  $^1\text{H}$  qNMR even complements X-ray diffraction because NH and O fragments can hardly be distinguished from one another in the diffraction pattern, while hydrogen in hydrogen-bonds often results in exceptional chemical shift values (10–18 ppm [18–21]) which helps spectral resolution and assignment in the NMR spectrum.

Systematic studies in solid-state qNMR have highlighted different sources of errors, for example repetition delays (related to spin-lattice relaxation) [3,9], pulse-length effects [9], spectrometer stability issues [15], sample preparation, quantitative analysis with internal or external reference [2,3,14,15] and packing effects of the material in the MAS rotor [14,15], which lead to false intensities when excited by inhomogeneous radio-frequency fields. An exhaustive protocol for solid-state qNMR has recently been published [9].

As for other analytical methods also in qNMR many of the above mentioned problems can be circumvented by quantification relative to an internal reference [2,3], in contrast to quantification to an external reference, which critically depends on the stability of the spectrometer sensitivity constant. For this purpose the to-be-analyzed sample is mixed with a known reference sample of high purity (internal reference) where the mass ratio, reference

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mass  $m_{ref}$  to analyzed sample mass  $m_{sample}$ , is known. The area below a peak  $a$  is proportional to the number of nuclei detected by NMR. Consequently, the ratio of peak areas is proportional to the ratio of the number of detected nuclei  $n_{nuc}$  in mole in the analyte and the reference sample in the ideal case. Because the molar mass and the number of detected nuclei per mole  $X_{ref}$  of the reference sample is known, the ratio  $\frac{n_{nuc,analyte}}{n_{nuc,ref}}$  may be rewritten in the following form.

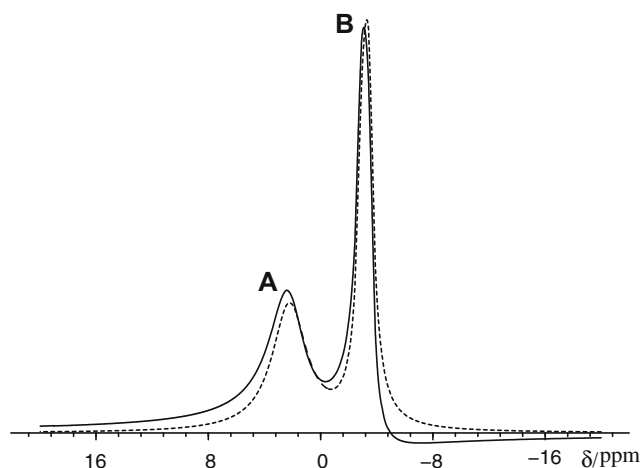
$$\frac{a_{analyte}}{a_{ref}} = \frac{n_{nuc,analyte}}{n_{nuc,ref}} = \frac{n_{nuc,analyte}}{n_{ref}X_{ref}} = \frac{n_{nuc,analyte}}{m_{ref}/M_{ref}X_{ref}} \quad (1)$$

Variations in natural abundance of the detected isotope have been neglected for simplicity. The result of a quantification is typically given in mole analyte per mass  $\frac{n_{nuc,analyte}}{m_{sample}}$ . Its calculation from Eq. (1) is straight forward. In our experience the usage of an internal reference improves the relative error of  $\frac{n_{nuc,analyte}}{m_{sample}}$  from about 30% for quantification to an external reference to below 10% for spin-1/2 nuclei other than  $^{19}\text{F}$  and  $^1\text{H}$  (results not shown).

Because our first results of solid-state  $^1\text{H}$  qNMR on amorphous samples were disappointing, here we present an analysis of possible errors and an improved protocol. Our hypothesis is that the combination of receiver dead time delay, homonuclear dipolar dephasing and baseline distortions leads to frequency dependent phase shifts of the signal which cause significant errors in qNMR with internal references. To test the hypothesis we derive an analytical expression for the relative error of the peak area ratio of two dephased peaks of Lorentzian lineshape. We then propose a protocol based on an extrapolation approach and the Hahn echo [22] which avoids the receiver dead time and accounts for intensity losses through homonuclear dipolar dephasing and transversal relaxation. Finally, the protocol is validated for a sample with strong  $^1\text{H}$ - $^1\text{H}$  magnetic dipole-dipole couplings at low magnetic fields and moderate sample spinning frequencies and compared to data obtained at medium magnetic fields and very high sample spinning frequencies.

## 2. Theory

The hypothesis of this contribution is that even when following the protocol presented in Ref. [9,14] phase errors may distort the lineshape of a neighboring peak to such an extent that in case of broad, poorly resolved peaks a significant error in the determined peak area ratio may result. A visual presentation of the idea is presented in Fig. 1. A small phase error will cause an admixture of dis-



**Fig. 1.** Simulated NMR spectra of a line shape function perfectly in phase (dotted line) and a lineshape being out of phase (signal phase  $\phi = 10^\circ$ ) but with otherwise identical lineshape parameters.

persive lineshape of peak A to peak B so that amplitude of peak B is virtually decreased while in reverse peak B adds a bit of intensity to peak A. We conclude that the resulting error in peak ratio  $r$  depends on the signal phase  $\phi$  and may be positive or negative. Because dispersive lineshapes have a much slower decaying behavior away from the center frequency than their absorptive counterparts, it is interesting to ask in which cases this mechanism is relevant. In the following we derive an estimate of this error based on Lorentzian lineshapes.

To derive a simple analytical expression of the relative error  $\frac{\Delta r}{r}$  of the peak ratio  $r$  which results from a dephased spectrum of two peak A and B, we need to make a number of simplifying assumptions. We assume the two peaks can be described as Lorentzian functions with the parameters integrated peak area  $a$ , linewidth (full width at half height in Hz)  $\lambda_i$  and center frequency  $\nu_i$  for peak  $i$ . The real part of complex Lorentzian [23]  $L_i(\nu, \nu_i, \lambda_i, a_i, \phi)$  may be written in terms of an absorptive  $L_{abs,i}(\nu, \nu_i, \lambda_i)$  and a dispersive contribution  $L_{dis,i}(\nu, \nu_i, \lambda_i)$ .

$$L_{abs,i}(\nu, \nu_i, \lambda_i) = \frac{\lambda_i/2}{\pi((\lambda_i/2)^2 + (\nu - \nu_i)^2)} \quad (2)$$

$$L_{dip,i}(\nu, \nu_i, \lambda_i) = \frac{-(\nu - \nu_i)}{\pi((\lambda_i/2)^2 + (\nu - \nu_i)^2)}$$

The spectral lineshape of a resonance with a phase  $\phi$  can be determined by evaluating the following function over all frequencies  $\nu$ . A peak in pure absorption then has a phase of  $0^\circ$ .

$$L_i(\nu, \nu_i, \lambda_i, a_i, \phi) = a_i(\cos(\phi)L_{abs,i}(\nu, \nu_i, \lambda_i) - \sin(\phi)L_{dis,i}(\nu, \nu_i, \lambda_i)) \quad (3)$$

The total lineshape  $S_{exp}(\nu)$  of a dephased spectrum consisting of two peaks can be written as follows.

$$S_{exp}(\nu) = L_A(\nu, \nu_A, \lambda_A, a_A, \phi) + L_B(\nu, \nu_B, \lambda_B, a_B, \phi) \quad (4)$$

In order to deconvolute such a spectrum we assume it is being fitted with two Lorentzian lineshapes in pure absorption, i.e.  $\phi = 0$ , where linewidths and center frequencies do not change.

$$S_{fit}(\nu) = L_{abs,A}(\nu, \nu_A, \lambda_A, a_{Afit}) + L_{abs,B}(\nu, \nu_B, \lambda_B, a_{Bfit}) \quad (5)$$

The task is to find the fitted peak areas  $a_{Afit}$ ,  $a_{Bfit}$  which give the smallest deviation between  $S_{fit}(\nu)$  and  $S_{exp}(\nu)$  in other words the smallest “total square error”  $E$ .

$$E = \int_{-\infty}^{+\infty} (S_{exp} - S_{fit})^2 d\nu \quad (6)$$

Necessary conditions for the optimum set of parameters  $a_{Afit}$ ,  $a_{Bfit}$  are

$$\frac{\partial E}{\partial a_{Afit}} = 0 \quad \wedge \quad \frac{\partial E}{\partial a_{Bfit}} = 0 \quad (7)$$

These two equations can be solved simultaneously for  $a_{Afit}$  and  $a_{Bfit}$ , the term  $\nu_A - \nu_B$  is substituted by  $\Delta\nu$ , the sum of linewidths  $\lambda_A + \lambda_B$  by  $\lambda_s$ , the term  $a_A/a_B$  by  $r$  and the relative error  $\frac{\Delta r}{r} = \frac{a_{Afit}/a_{Bfit} - a_A/a_B}{a_A/a_B}$  of the ratio is calculated in Eq. (8). Because experimental spectra are usually “phased” such that the phase  $\phi$  is very close to zero a Taylor expansion up to second order of  $\phi$  around  $0^\circ$  may be applied to identify the main sources of error.

$$\frac{\Delta r}{r} \approx X_1 \cdot \phi + X_2 \cdot \phi^2 \quad (8)$$

where  $X_1$  and  $X_2$  (see Supporting information) are

$$X_1 = \frac{4\lambda_s(\lambda_A\lambda_s + 4\lambda_A\lambda_B r + \lambda_B\lambda_s r^2)\Delta\nu + 16(\lambda_A + \lambda_B r^2)\Delta\nu^3}{r((\lambda_A^2 - \lambda_B^2)^2 + 8\lambda_s^2\Delta\nu^2 + 16\Delta\nu^4)} \quad (9)$$

This formula provides an error estimate for a given set of line shape parameters of two peaks. We calculated plots of the relative error  $\Delta r/r$  as a function of the signal phase  $\phi$  for a particular intensity ra-

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