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Precise NMR Method for Titering Organometal Reagents

Vineet K. Jakhar, Eric C. Johnson, Aditya Kavuturu, Jessica K. Heller, Adam S. Veige, and Ion Ghiviriga*

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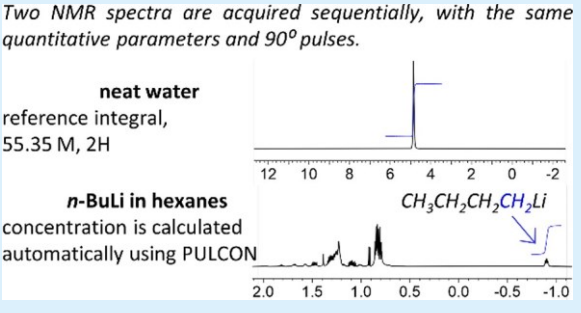
ABSTRACT: The concentration of organometal reagents can be conveniently determined by obtaining the NMR spectra of the neat reagent solution, and, in a second NMR tube, of a neat reference solvent. The PULCON relationship, implemented in all major NMR software, is then used to calculate the concentration of the reagent based on the absolute integrals in the spectra, the known concentration of the reference, and the number of protons under the integrals.



Used as strong bases and/or nucleophiles, organometal reagents, such as *n*-butyllithium, *t*-butyllithium, lithium diisopropylamide, and corresponding organomagnesium compounds, are ubiquitous in modern chemical synthesis. Purchased as solutions in an appropriate inert solvent, their utility relies on an accurate evaluation of their concentration. Unlikely at any point in their lifecycle to reflect the manufacturer's labeled concentration, organometal reagents are vulnerable to moisture and air, improper storage, solvent evaporation, and thermal stability. Often generated *in situ*, measuring the concentration of organomagnesium reagents must occur prior to use, or during their preparation quantitative conversion is assumed. Considering that reagent utility centers on accurate concentrations, it is important to periodically, if not prior to every use, evaluate the concentration by some form of titration. Numerous experimental titrating methods[1] for determining the concentration of organometal reagents provide satisfactory results, although the time-consuming and tedious task discourages routine evaluation. Another drawback is that most experimental methods use specific indicators for certain reagent/solvent pairs, and importantly, the end-point observation is prone to operator error. Perhaps most damning of all is the notion that experimental titration provides absolute concentrations. Practitioners place unreasonable confidence in experimental titration revealing the truth. Mistaken as accuracy, titrating multiple times to provide an average captures inherent errors and only provides precision. A longstanding challenge is to develop a protocol for evaluating the absolute concentration of organometal reagents rapidly, accurately, and with ease.

Nuclear magnetic resonance (NMR) is intrinsically quantitative, and under appropriate acquisition conditions,[2] the accuracy of the method is <1%. A point that is often overlooked is that the limit for precision of quantitation must be set because full relaxation of the NMR nuclei after a pulse theoretically takes infinite time, and to capture the whole area of a Lorentzian signal, the integral must cover the whole, infinite range of frequencies. The conditions for accuracy of 1% are (i) the repetition rate of the 90° pulses must be larger than 4.6 × T1, and (ii) the integration must cover 25 line widths at half-height in each direction. In practice, for signals with similar widths, smaller integral regions are often used. In short, achieving 1% accuracy requires well-separated signals and a sufficiently long relaxation delay.

For organometal reagents, the use of inert internal standards such as benzene and 1,5-cyclooctadiene (COD) has been reported.[3] The method involves weighing the standard and then mixing it with a precisely measured volume of reagent solution. Using parameters appropriate for quantitation, the user records an NMR spectrum in nondeuterated solvent conditions, without lock (no-D NMR).[4] Another recently published method eliminates the need for weighing the standard for each sample and also allows for the field-frequency lock by sealing a solution of the reference compound and the deuterated lock solvent in the exterior space of a concentric tubes device.[5] Although there are plenty of choices for a reference, matching to the analyte requires at least one signal in each to be free from overlap; therefore, the use of an internal reference will never be a generalized approach in any of these methods.

We propose here a new NMR method for titering organometal reagent solutions that removes the need to mix, weigh, or measure reagent volumes. The method only requires

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placing the reagent solution and a neat solvent into separate NMR tubes and measuring the two spectra, with acquisition parameters adequate for quantitation. A *fl*owchart summarizing the procedure is presented in Figure 1. Contained in the

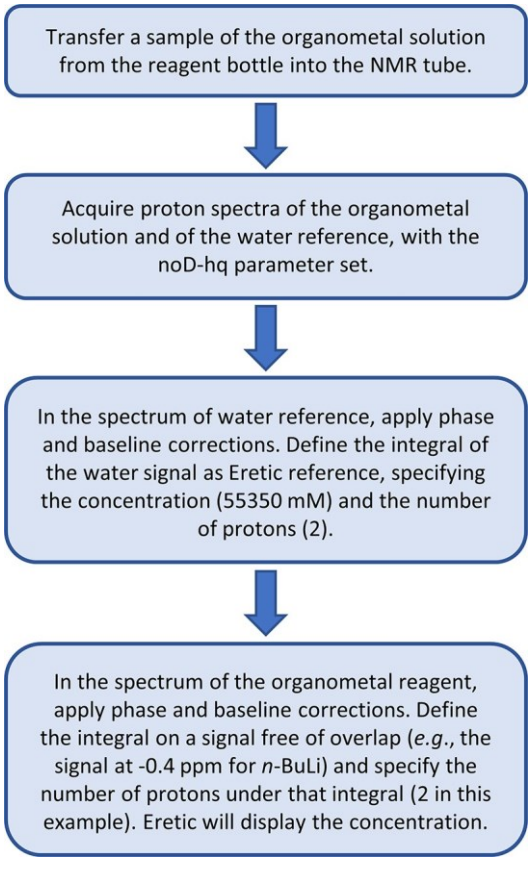

Figure 1. Flowchart describing the method.

Supporting Information (SI) are the parameter set and the instructions to implement the method. The method's fundamental concept is to relate the unknown concentration of the reagent solution to the neat reference's known concentration with the support of a quantitation module implemented in all major NMR software, for example, ERETIC2 in Topspin.

Using an external standard eliminates signal overlap and side reactions with the analyte. However, acquiring two spectra requires corrections to account for the di*ff*erent conditions between the two samples, even when using the same acquisition parameters. For example, using the same parameters on tuned and matched samples, a value that is 25% lower than the expected value results when measuring the concentration of isopropanol with water as the reference. The lower value comes from the fact that a more ionic sample absorbs more of the radio frequency (RF) energy from the transmitter coil as an excitation pulse, translating to a longer 90° pulse. Similarly, the sample will absorb more of the RF signal coming from the protons into the receiver coil, leading to a less intense signal in the spectrum. Fortunately, the reciprocity principle states that when using the same coil as a transmitter and a receiver, as in Fourier transform (FT)-NMR, the observed signal area is proportional to the reciprocal of the 90° pulse.[6] The PULCON (pulse length-based concentration determination) relationship[7] (eq 1) quantitatively relates integrals in spectra of samples that absorb RF di*ff*erently

$$[\text{analyte}] = (A_{\text{analyte}}/\text{NS}_{\text{analyte}})/(A_{\text{reference}}/\text{NS}_{\text{reference}}) \times (\text{pw90}_{\text{analyte}}/\text{pw90}_{\text{reference}}) \times [\text{reference}] \quad (1)$$

where [ ] represents the molar concentration, A is the area of the signal, NS is the number of spins responsible for the area, and pw90 is the 90° pulse.

The parameter set in the SI contains an automation macro that measures the 90° pulse and uses this value for the excitation pulse. ERETIC2 automatically reads the pulse width values (pw90) from the data sets and the absolute integrals (A) from the spectra and requires an input of the reference's concentration and the number of spins under the integrals.

The solvent volume di*ff*erence between the reference and analyte samples can also lower the accuracy. It is important to use NMR tubes from the same manufacturer and the same model when performing any analytical NMR experiment. The height of the sample must cover the area sensed by the receiver coil; 50 mm works for most probes. Di*ff*erences in the internal diameter of the NMR tubes lead to errors up to 7%. When using tubes of the same model/manufacturer, these di*ff*erences fall into the error range goal of 1%. For example, measuring the water concentration in 24 Wilmad 507-PP-7 tubes reveals an average inner diameter of 4.2065 ± 0.0065 mm corresponding to an inherent volume variation of only ±0.3%.

Neat liquids have molar concentrations near 10 M, making them appropriate references for typical organometal reagents that often have concentrations in the range of 1−3 M. In fact, by using modern instruments that sample the free induction decay (FID) at the maximum possible rate (oversampling) and then apply decimation to reduce the number of points to the Nyquist rate, neat solvents can cover a wider range of concentrations. The process involves averaging, which increases the e*ff*ective digital resolution of the analog-to-digital converter (ADC) for typical proton spectra to 22 bits. Ignoring limitations from signal-to-noise ratio, a precision of 1% corresponds to an intensity ratio of $2^{22}/100 = 42\,000$. Supporting the claim of 1% precision, the SI contains experimental data of dioXane concentration measurements in toluene using a water reference. Remarkably, concentrations as low as 10 mM are possible with this method. For perspective, the concentration of residual chloroform in 99.8% chloroform-*d* is 25 mM.

One aspect to consider whenever acquiring the spectra of neat liquids and solutions of organometal reagents is the e*ff*ects of radiation damping,[8] namely, broad lines for the intense signals and phase distortions of the multiplets. The strong FID in the receiver coil acts as a selective pulse, which returns the magnetization to the z axis. The frequencies of the strong lines decay fast; therefore, their signals in the spectrum are broad. These signals will have the correct intensities in the spectrum, but automated phase adjustment software might not function properly.

Evaluated in this study are nine organometal reagent solution concentrations using a water sample as an external reference and the PULCON method. Concentrations measured (5×) in a nitrogen atmosphere gloveboX of the same reagent bottles, during the same day, using COD as an internal standard,[3d] and by two di*ff*erent titration methods[3d,1p] serve to compare with the NMR method. Table 1 lists the results and

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Table 1. Concentration of Organometal Reagents[a]

reagent	[COD]	[water]	[titr. 1]	[titr. 2]	% err. water	% err. titr. 1	% err. titr. 2
^{n}BuLi 2.5 M in hexanes	2.47	2.51	2.65	2.35	1.6	7.1	−5.2
^{t}BuLi 1.7 M in pentane	1.61	1.69	1.75	1.55	5.3	8.8	−3.5
LDA 1.0 M in THF/hexanes	0.87	0.90	1.04	0.95	3.2	19.8	9.5
MeLi 1.6 M in Et_2O	0.55	0.59	0.64	0.61	6.7	15.6	10.2
EtLi 0.5 M in benzene/cyclohexane	0.53	0.53	0.60	0.55	−1.0	13.1	2.3
EtMgBr 3.0 M in Et_2O	3.07	3.20	3.16	2.90	4.3	3.2	−5.5
VnMgBr 1.0 M in THF	0.92	0.92	1.10	1.00	−0.1	19.5	8.7
AllylMgCl 2.0 M in THF	2.35	2.25	2.40	2.19	−4.2	2.4	−6.5
PhMgBr 3.0 M in Et_2O	2.97	3.01	3.10	2.80	1.4	4.3	−5.9

[a][COD], molar concentration determined using 1,5-cyclooctadiene as an internal reference;[3d] [water], determined using a water sample as an external reference; [titr. 1], determined by titration with *l*-menthol using 2,2′-bipyridine as an indicator;[3d] [titr. 2], determined by titration with 2-hydroxybenzaldehyde phenylhydrazone as an indicator.[1p] The % errors are relative to the internal standard [COD] method.

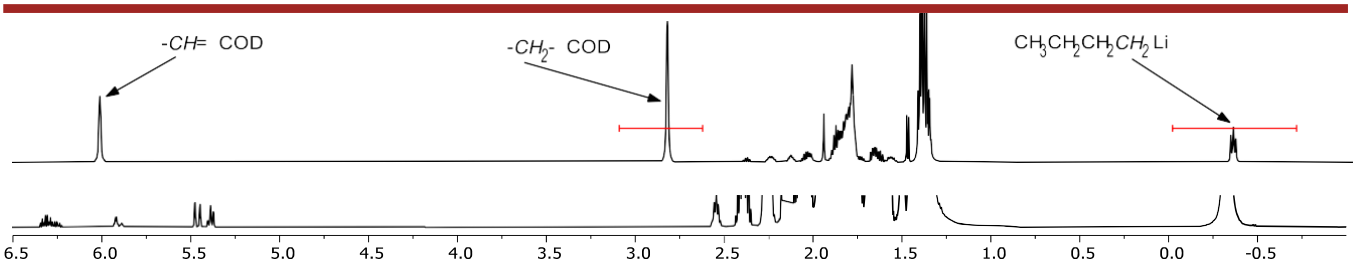

Figure 2. Proton spectra (with ^{13}C decoupling) of *n*-butyllithium in hexanes. The top spectrum is of the sample used to determine the concentration using COD as an internal reference. The integral regions used for calculation are marked in red. The bottom spectrum is of the neat organometal solution used to determine the concentration using water as an external reference. The intensity was adjusted for the signals of the impurities to identify regions free of overlap.

reports errors relative to the internal standard method. Standard deviations, given in the SI, are ∼1% for all methods.

NMR methods are accurate if the signals chosen for quantitation are not overlapping with other signals. As observed for *n*-butyllithium (Figure 2) and other reagents (Figure S1), many organometal reagents have protons that resonate in the upfield region of the spectrum. The spectrum of the neat reagent solution, plotted with high intensity in the bottom part of Figure 1, reveals inherent impurities between 5.4 and 6.4 ppm that overlap the COD alkene protons. This unforeseen overlap is a common problem for traditional NMR methods that employ an internal reference. Employing a neat liquid as an external standard avoids this complication. The *alpha* methylene protons for *n*-butyllithium are well-separated at −0.4 ppm. The PULCON and the internal standard methods are in excellent agreement for *n*-butyllithium, 1.6%, considering that the volume measurement in preparing the internal standard has a precision of 2% at best. For the other organometal reagents, the NMR methods differ by <5%, and again, we assign this to the error in the volume measurement for the internal standard. Surprisingly, quantitation differences between titrations and the COD method can be 20%, and those between the two titration methods can be as large as 10%. The most likely source for these errors is the presence of other strongly basic compounds in the reagent solution. Two different operators came to essentially the same results when titrations were performed for three organolithium reagents.

Estimating the accuracy of our external standard method by comparing the results to those of the internal standard method is limited by the errors inherent in the latter. Neat solvents offer samples of precisely known concentrations, calculated from molar weigh and density. The concentrations measured on 20 neat solvents using PULCON (Table S1) demonstrate that the accuracy of our method is 1%, similar to that obtained for external standards in deuterated solvents.[9]

Reliant on internal references, previously developed NMR methods to titrate organometal solutions require sample preparations by either volumetric or gravimetric methods, thus adding layers of inconvenience and intrinsic error. As demonstrated for the first time, it is now possible to simply relate an external reference solvent signal to the analyte signal of interest using eq 1. The method is easy. The only preparation involves transferring the reagent solution of interest and the reference solvent into NMR tubes. The rapid evaluation and elimination of the gravimetric or volumetric measurements of the analyte or reference reagent will greatly facilitate organometal synthesis. Adding to the convenience, if sealed, use of the same reference solvent multiple times is possible, thus reducing the experiment to simply placing an aliquot of the reagent into an NMR tube and acquiring the two spectra. Most synthetic laboratories contain multiple bottles of organometal reagents containing unknown concentrations of the reagent. Evaluating the organometal concentration by wet-lab titration methods is arduous, time-consuming, and prone to user error, leading to the propensity to simply buy a new bottle. Hopefully, this method removes the barrier and leads to routine testing. We created a general setup for quantitation by NMR, which can be applied to any task requiring knowledge of concentration, for example, purity determination or the measurement of equilibrium, rate, or association constants.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c01006.

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Instructions on how to install the necessary files in Topspin, how to run the experiments, and how to process the data (PDF)
Files to be installed in Topspin (ZIP)
Video tutorial on how to run the experiments (MP4)
Video tutorial on how to process the data (MP4)
FAIR data, including the primary NMR FID files, for reagents (ZIP)

AUTHOR INFORMATION

Corresponding Author

Ion Ghiviriga − *Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, United States;* orcid.org/0000-0001-5812-5170; Email: ion@ufl.edu

Authors

Vineet K. Jakhar − *Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, United States*
Eric C. Johnson − *Bruker Biospin, San Jose, California 95134, United States*
Aditya Kavuturu − *Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, United States*
Jessica K. Heller − *Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, United States*
Adam S. Veige − *Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, United States;* orcid.org/0000-0002-7020-9251

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.1c01006

Author Contributions

The manuscript was written through the contributions of all authors. I.G. designed the method and tested the initial NMR experiments. V.K.J., A.K., and J.K.H performed the titrations and the NMR measurements. E.C.J. wrote the automation program for measuring the 90° pulse. I.G. and A.S.V. prepared the manuscript.

Notes

The authors declare no competing financial interest.

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