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Precision and sensitivity optimization of quantitative measurements in solid state NMR

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Abstract

This work presents a methodology for optimizing the precision, accuracy and sensitivity of quantitative solid state NMR measurements based on the external reference method. It is shown that the sample must be exclusively located within and completely span the coil region where the NMR response is directly proportional to the sample amount. We describe two methods to determine this "quantitative" coil volume, based on whether the probe is equipped or not with a gradient coil. In addition, to improve the sensitivity and the accuracy, an optimum rotor packing design is described, which allows the sample volume of the rotor to be matched to the quantitative coil volume. Experiments conducted on adamantane and NaCl, which are representative of a soft and hard material, respectively, show that one order of magnitude increase in experimental precision can be achieved with this methodology. Interestingly, the precision can be further improved by using the ERETIC™ method in order to compensate for most instrumental instabilities.

Keywords: Solid state NMR; HR MAS; Quantitative NMR; Optimized rotor packing; ERETIC™

1. Introduction

Nuclear Magnetic Resonance (NMR) is a well established technique for characterizing the structure and dynamics of all kinds of physicochemical systems, both in the liquid and the solid state. NMR is also commonly used for quantitative measurements, especially in the liquid state. Recently, solid state NMR (ssNMR) has become increasingly popular for achieving quantitative determinations. In fact, ssNMR is a non-destructive, non-invasive and rather versatile technique, which allows both heterogeneous and amorphous materials as well as homogeneous crystalline phases to be studied.

Basically, quantitative determinations in the solid state rely on the use of Single Pulse Excitation (SPE) Magic Angle Spinning (MAS) or Cross Polarization (CP) MAS experiments. However, because of the complexity of cross polarization dynamics [1], quantitative determinations achieved by CP MAS experiments are not trivial [2–4], and hence SPE MAS experiments are usually preferred, provided of course that sensitivity is not an issue. A few quantitative studies in the solid state have been reported so far [5], and the investigated systems have been as diverse as, for instance, drugs [6–8], cement based materials [9,10], soils and humic substances [11,12], coals [13] and resin loadings [14]. As a matter of fact, recent technological advances and the currently less prohibitive price of the required instrumentation have made quantitative ssNMR available to chemists and biochemists as a new analytical tool.

As a preliminary step, quantitative determinations in ssNMR obviously require optimal spectrometer settings. In this context, Harris et al. [6] have recently proposed an exhaustive protocol to control and optimize the acquisition and processing parameters of quantitative ssNMR

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data. Although this particular protocol has been devised for measurements of formulated systems, it may easily serve as a basis to build more generally applicable quantification procedures, which in turn depend on the analytical problem to be solved [15]. Typically, most absolute quantitative measurements rely on the use either of an internal or an external standard, even though hybrid solutions have also been described.

In liquid state NMR, the internal reference method is commonly used [15], although applications of the external reference method can also be found [16]. The major difficulty of the internal reference method lies in the selection of an adequate internal standard. Obviously, this compound should be soluble in the solvent of choice, stable and inert with respect to the analyte. In addition, it should ideally be characterized by only one NMR signal, a singlet being best, which should clearly not overlap with the analyte signals. This NMR signal should also be characterized by a relatively short T_1 value, at least equal to those of the analyte signals, in order to avoid fruitlessly increasing the experimental time.

In contrast, although the use of an internal reference in ssNMR avoids some experimental complications (e.g. variable sample volumes or B_1 inhomogeneity) [6], this method remains typically more difficult to implement, mostly because of the complexity to prepare a perfectly homogeneous mixture of the sample and the reference. Therefore, in this case, the external reference method is usually preferred, also because it offers several specific advantages. First, the calibration can be made by analyzing the analyte itself or, when unavailable, similar compounds readily available. Second, the fact that no exogenous chemical is introduced in the rotor has the double advantage of maximizing the experimental sensitivity and of allowing recovery of the sample, in the case it is precious or further analysis is required.

Unfortunately, the external reference method also has significant drawbacks. Indeed, its precision strongly depends on the overall instrumental stability. Whether they originate in the probe or in the spectrometer, instrumental instabilities, especially in electronics, may randomly perturb the outcome of successive experiments. This in turn implies significant loss of experimental precision. The effect of such instabilities can be somehow reduced by using the ERETIC™ method [17]. While being initially devised for liquid state NMR experiments [18], this method has recently been adapted and successfully applied in the solid state [19]. In brief, a low-power RF shaped pulse mimicking the form of a NMR signal is applied during the acquisition time. After Fourier Transform of the time domain signal, a reference peak appears at a frequency chosen by the operator. The relative intensity of this peak can be adjusted by changing the corresponding pulse power level. As this pulse experiences all the detection side of the circuit, its intensity variation will monitor all instabilities due to this particular part of the electronics and can hence be used to correct the intensity of NMR peaks arising from the sample, which are affected by the very same perturbations.

Another source of error comes from the possible lack of a quantitative response along the whole volume of the sample holder, i.e. the rotor. Namely, the sample region inside the rotor itself in which the linear relationship between the intensity of the detected NMR signal and the weight of the analyzed compound holds (the ''quantitative volume'') is a function of the circuit geometry and may be smaller than the volume submitted to the analysis. In fact, while the signal in liquid state NMR is related to the analyte concentration, in ssNMR it relates to an absolute measurement of the amount of nuclei, which in turn relates to the sample weight. As the ''quantitative volume'' and the rotor volume are not necessarily the same, the signal intensity of a given set of nuclei does not only depend on the amount of spins but also on their position within the rotor. In other words, for a given sample weight, the resulting signal intensity that is measured will depend on the sample distribution within the rotor volume with respect to the corresponding quantitative volume. Because this distribution strongly relates to the physical characteristics of the sample itself (specific weight, granulometry, hardness...), errors can easily come along. Specifically, the sample preparation may clearly influence the results of the quantitative analysis, especially because there is also a strong dependence on the analyst skills.

To reduce this type of error, it is necessary to ensure that the whole sample actually fits into the part of the rotor volume that is coincident with the quantitative volume. This quantitative volume mainly depends on the coil geometry. Obviously, the number of coil turns is a preponderant factor, as the quantitative volumes of 8-turn and 12-turn coils are necessarily different. However, even for coils having the same number of turns, small changes in coil geometry may still lead to large changes for the quantitative volume.

Therefore, the quantitative volume of a given probe should clearly be calibrated as a very first step in any quantitative measurement by ssNMR. In this context we propose a methodology that should be applied whenever quantitative determinations based on the external reference method are to be performed in ssNMR. This methodology aims at determining the quantitative probe volume by using two distinct methods, with or without using magnetic field gradients. In addition, we introduce a specifically designed rotor prototype to increase the sensitivity by matching the effective sample volume of the rotor to the quantitative volume. We also show that further improvement in precision can be gained with the aid of the solid state ERETIC™ method.

2. Experimental

2.1. Samples

Two samples were used in this study: adamantane and NaCl. The adamantane (purum, 99.0% GC) and NaCl

(puriss., 99.94% AT) samples were purchased from Fluka and Merck, respectively. In addition, a D_2O sample (Eurisotop, 99.8%) doped with $CuSO_4$ was used for establishing the B_1 profile of the HR MAS probe with the gradient based method. All materials were used as received.

2.2. NMR

All NMR experiments were conducted at 300 K on a BRUKER AVANCE400 DPX and BRUKER AVANCE400 DSX spectrometers, both operating at 400 MHz for the ^1H Larmor frequency. These spectrometers were equipped with a double resonance $^1H/^{13}C$ HR MAS and a $^1H/X$ CP MAS probe, respectively. The HR MAS probe was also equipped with a ^2H lock channel and a magic angle gradient coil. For the MAS experiments recorded on the adamantane and NaCl samples, the spinning rate was set to 4 kHz and 10 kHz, respectively. These experiments used a Single Pulse Excitation (SPE) pulse sequence. The relaxation delays were chosen to ensure complete relaxation; an interpulse delay of 15 s and 60 s were used for adamantane and NaCl, respectively. In all experiments the number of scans was optimized to achieve a signal-to-noise ratio higher than 150 in order to minimize the experimental uncertainty due to the noise level (below 1%) [3]. All commercial rotors were provided by BRUKER.

2.3. Excitation profiles

For the gradient based method, a 100 μl rotor filled with the doped D_2O sample and the pulse sequence described by Hurd et al. [20] were used. Note that the sample was placed at the magic angle but not spun. The pulse sequence consists of a simple Hahn echo in which the 180 pulse is flanked by two gradient pulses (g) and the echo is acquired in the presence of another gradient (g_a). The EXORCYCLE and CYCLOPS phase cycles were used. The echo time was set to 27 ms. The duration of the two central gradient pulses g was 2 ms and the time between them was 40 ms. An acquisition time (aq) of 6.5 ms was used. The strengths of the rectangular gradients g and g_a were about 3.5 G cm^{-1} and 2.9 G cm^{-1}, respectively. Importantly, to place the echo time in the middle of the acquisition period, a negative rectangular gradient twice as strong as the acquisition gradient (5.8 G cm^{-1}) but with a duration equal to aq/4, was applied just before starting the acquisition. Thirty-two transients composed of 256 complex data points were acquired. The data were processed in the magnitude mode without using apodization.

For the method based on successive sample additions, 4.0 mg of adamantane were placed inside a 100 μl rotor and firmly packed. This weight was chosen to ensure a sample slice thickness of about 0.8 mm. Subsequently, a ^1H and ^{13}C SPE MAS experiment was recorded for the HR MAS and CP MAS probe, respectively. This procedure was repeated 20 times until the rotor was full. For the profile of the HR MAS probe (^1H observation), the adamantane sample was placed at the magic angle without spinning whereas, for the CP MAS probe (^{13}C observation), the spinning rate was set to 4 kHz.

2.4. Data processing

After careful manual phase and baseline corrections, the intensities were determined by integrating the signal areas in a region extended at 30 times the line-width (about 70 Hz) each side of the peak [21]. To evaluate the standard deviations, the intensities were either used as such or normalized to the intensity of the ERETIC™ signal. For all the profiles determined with the successive addition method, the error bars of the data points are included within the symbols (either squares or circles).

3. Results and discussion

3.1. Determination of the probe quantitative volume

The determination of the probe quantitative volume is related to the determination of the excitation field (B_1) profile of the probe. Typical methods to obtain such profile use magnetic field gradients. As such, an elegant piece of work has recently shown that a complete three dimensional map of the B_1 field could be obtained by using a three axis gradient coil and a dedicated pulse sequence [22]. Alternatively, due to basic symmetry considerations, the probe quantitative volume can simply be inferred from the projection of the B_1 field profile along the rotor axis. At least two methods can be used to do so.

A first possible approach makes use of a magnetic field gradient aligned along the rotor axis, sometimes called magic angle gradient [23]. As emphasized by Hurd et al. [20], a crude measure of the B_1 field homogeneity along the length of the coil can simply be obtained by recording an image of the sample along the gradient axis with a Hahn echo acquired in the presence of a magnetic field gradient.

Another method consists of recording a series of NMR spectra on a given sample, for which identical amounts of material are successively added inside the rotor. Specifically, an amount of sample, precisely weighed, is introduced inside the rotor and the sample is firmly packed. Then, the thickness of the resulting sample slice (about 1 mm) is measured with a graduated cylinder, and a SPE MAS NMR experiment is recorded. This procedure is repeated until the rotor is completely full. In the end, by plotting the difference in signal intensity obtained for two successive experiments as a function of the sample slice thickness, the projection of the B_1 field profile along the magic angle can be determined. As a sample, we recommend the use of adamantane because it exhibits relatively short T_1 relaxation times and allows ^{13}C SPE MAS experiments to be recorded with a good sensitivity. In addition, to compensate for any instrumental instability in the spectrometer electronics, the ERETIC™ method may be used,

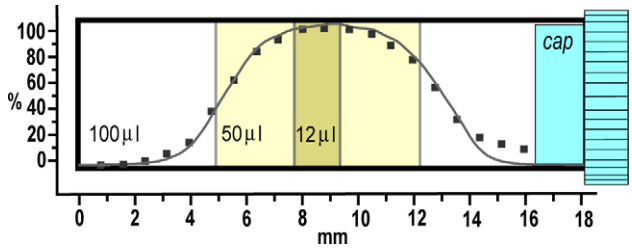

Fig. 1. Comparison of the B_1 field profiles obtained at 400 MHz on a 4 mm HR MAS probe equipped with a magic angle gradient coil and an 8-turn receiver coil. The straight line and the filled squares illustrate the profiles obtained with the gradient based method and the successive sample addition method, respectively. In the latter case, instrumental instabilities were compensated using ERETIC™. The sample volumes corresponding to commonly used 4 mm rotors (12, 50 and 100 µl) are also shown.

by normalizing the intensities (integrals) of the adamantane signals to the integral of the ERETIC™ signal [19].

The profiles achieved by both methods are shown in Fig. 1. As expected, completely equivalent profiles are obtained. In this case, the experiments were performed on a 4 mm HR MAS probe equipped with a gradient coil aligned along the magic angle axis and an 8-turn receiver coil.

Whenever a gradient coil is available, the first method should obviously be used because it is much faster. However, when such a coil is unavailable, the second method is shown here to provide also a reliable estimate of the quantitative volume. This method has the additional advantage that it can be implemented on any standard CP MAS probe. Finally, while the precision of the gradient method relates to the field-of-view chosen to record the experiment, the precision of the latter method directly relates to the thickness of the sample additions that were used for establishing the profile.

3.2. Optimized rotor packing

As we have shown in the previous section, the quantitative volume may vary as a function of the experimental design and of the used equipment. Sample restraint into the coil quantitative volume is easily obtained by using commercial rotors of limited loading. However, this solution is far from optimum, as it follows a conservative choice of the rotor geometry, compatible with all possible experimental situations. Maximum sensitivity is sacrificed for robustness. As the objective of quantitative analysis is the detection of percent components, for example in pharmaceutical formulations, sensitivity becomes a relevant issue to optimize, while keeping the most precise response. Following our previous discussion, it becomes clear that for a given experiment, methods have to be developed for limiting the rotor volume as close as possible to the quantitative volume. For different reasons, a similar problem has been faced while seeking for best conditions in multiple-pulse line-narrowing in proton MAS spectroscopy. In that situation, B_1 variations translated in uncontrolled and unwanted effects, and a consequent degradation in the sought line narrowing.

To cope with this issue, two approaches have been proposed: physical limitation of the sample by using inserts [24] and gradient selection of the desired sample portion [25]. Clearly, the gradient selection method does not address the sensitivity optimization issue. Moreover the gradient volume cut turns into a signal selection-through-destruction, which produces the same uncontrolled results as the regular full volume analysis. Namely, to produce sensible quantitative results, the weight of the analyte in the blanked area has to be assessed as precisely as the one in the quantitative volume. The physical volume selection is thus the only approach that can guarantee optimal NMR recording condition for solids. Optimized rotor packing should be achieved through a reasonably flexible approach, so that a rotor could be used for different occasions if required.

Although many different rotors are routinely available in a ssNMR laboratory, this work will focus for the purpose of the illustration on 4 mm rotors. However, all the following results and conclusions are clearly applicable to any rotor type.

Commercially available 4 mm rotors commonly have a sample volume ranging from 12 µl to 100 µl. Since typical quantitative volumes range from 20 µl to 30 µl (see Fig. 3), a 12 µl rotor is clearly a good candidate to ensure a fully quantitative response. However, this implies a great sensitivity penalty. Clearly, the rotor volume has to be adjusted to the quantitative volume in order to optimize the sensitivity.

Another point linked to the sample volume analyzed, and which is scarcely addressed in ssNMR, is its influence on the overall accuracy of the measurement. A mixed theoretical/phenomenological approach describing the general issue of sampling solids has been developed in the past years, providing estimates of the maximum expected accuracy that a given sampling procedure can provide, independently from the successive measurement errors. Details can be found in references [26–28], but a rather intuitive point stemming from this theory is that the accuracy of a measurement is linked to the faithfulness in the analyte concentration of the analyzed parcel with respect to the original lot. This is, in turn, proportional to the analyzed parcel size, and thus accuracy is expected to be best in ssNMR if the analyzed volume is maximized.

The rotor packing design proposed here allows the optimal filling condition to be matched. As shown in Fig. 2, we used a 4 mm o. d. Zirconia rotor, opened at both ends. Note in passing that this feature is especially convenient for rotor cleaning. Two Kel-F inserts, each equipped with two Viton o-rings, are used for closing. Most importantly, in order to match the effective sample volume of the rotor, 1 mm thick Kel-F discs, hereafter referred to as spacers, can be conveniently introduced inside the rotor. These spacers together with the inserts allow as much sample as

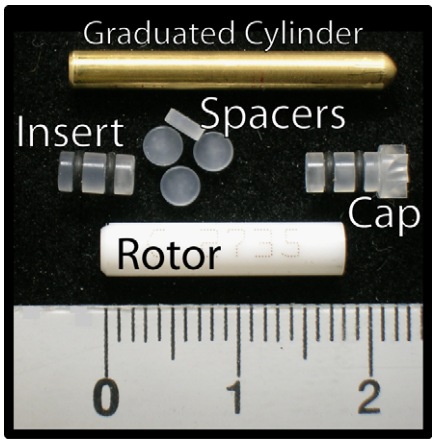

Fig. 2. Tools used for the packing design proposed in this work. A 4 mm o. d. Zirconia rotor opened at both ends was used. Closing is ensured by using, on one end, a Kel-F insert equipped with two Viton o-rings and, on the other end, the same insert plus a Kel-F cap. The 1 mm thick Kel-F disks (spacers) allow the available sample volume to be matched to the quantitative probe volume.

possible to be placed in the quantitative volume region of any probe.

3.3. Quantitative measurements

As a very first step, we have determined the B_1 field profiles of a CP MAS probe equipped with an 8-turn and a 12-turn receiver coil. Because no gradient coil was available, the previously outlined method based on successive sample additions was used. The results are reported in Fig. 3. As expected, comparison of the profiles shown in Fig. 3a and b confirms that the quantitative volume of a 12-turn coil is larger than that of a 8-turn coil. Fig. 3 also illustrates

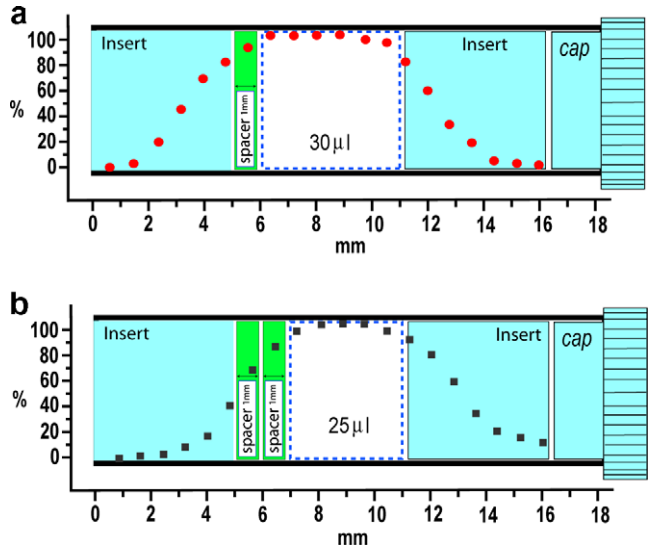

Fig. 3. Comparison of the B_1 field profiles obtained at 100.6 MHz on a 4 mm CP MAS probe equipped with (a) a 12-turn and (b) an 8-turn receiver coil. Both B_1 profiles were obtained with the successive sample addition method.

how, with the use of the rotor prototype depicted in Fig. 2, the effective sample volume of the rotor can be tailored to precisely match the probe quantitative volume. Specifically, one and two spacers were required in Fig. 3a and b, respectively.

In addition, comparison of Figs. 1 and 3 suggests that the sample volume of a 12 µl rotor clearly lies within the quantitative region of the probe, and hence this type of rotor could as well be used. However, this would lead to a severe sensitivity penalty, as more than half the available quantitative volume is unused. This is illustrated in Fig. 4. In Fig. 4a, the ^{13}C SPE MAS spectra of adamantane recorded with the 4 mm rotor prototype and the three commercial 100, 50 and 12 µl volume rotors are shown. The number of scans was the same for all experiments. The increase in signal-to-noise ratio achieved by using the prototype instead of the 12 µl rotor is clearly visible. Specifically, a factor of 2.5 in intensity is gained, which implies a reduction of a factor of 6 in experimental time for a same signal-to-noise ratio.

Similarly, Fig. 4b shows the ^{23}Na SPE MAS spectra obtained on a NaCl sample. The same observations hold. In addition, in both cases, it is immediately apparent that the increase in rotor volume, from 50 µl to 100 µl, is not accompanied by the theoretical factor 2 increase in signal intensity that should have been observed, had these volumes been included within the quantitative probe volume. Finally, note that the ERETIC™ method was used in all cases. This explains the presence of extra signals observed

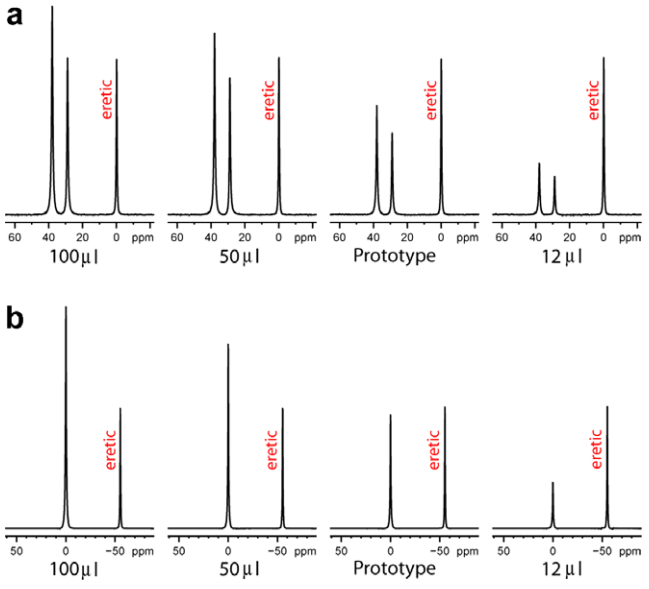

Fig. 4. (a) 100.6 MHz ^{13}C SPE MAS spectra recorded at 300 K with a spinning rate of 4 kHz on an adamantane sample in four distinct rotors; (b) 105.8 MHz ^{23}Na SPE MAS spectra recorded at 300 K with a spinning rate of 10 kHz on a NaCl sample in the same four rotors. The ERETIC™ signal appears as a singlet at 0 ppm and −50 ppm in (a) and (b), respectively. All ssNMR spectra were recorded on a 4 mm CP MAS probe equipped with a 12-turn detection coil.

at 0 ppm and −50 ppm in Fig. 4a and b, respectively, whose intensities are clearly constant.

To demonstrate the efficiency of the proposed methodology, we evaluated the repeatability of a single operator. Specifically, a given operator performed a series of five successive experiments with each of the four available rotors. Experiments were conducted both on adamantane and NaCl to illustrate the effects of the type of sample used; in fact, they are representative of a soft and hard material, respectively. For each of these five experiments, the same amount of sample was precisely weighted and put in the rotor. A ^{13}C and ^{23}Na SPE MAS experiment was then recorded for the adamantane and NaCl samples, respectively, on a 4 mm CP MAS probe equipped with a 12-turn receiver coil. The results are shown in Fig. 5, where the standard deviation obtained for each series is reported as a function of the type of rotor.

For adamantane, when going from a 50 μl or 100 μl rotor volume to an optimized rotor volume (30 μl), the standard deviation is reduced by a factor of almost 5 and 10, respectively. For NaCl, the standard deviation is reduced by a factor of 4 and 7, respectively. These relative reductions in standard deviation are expected to be even more significant for an 8-turn receiver coil because of its lower quantitative volume.

Moreover, the differences in precision evidenced between adamantane and NaCl for large rotor volumes illustrate the influence of the sample type on the outcome of quantitative measurements. Clearly, the softer the sample, the more difficult it is to control its distribution within the rotor volume. This is even more important when distinct operators prepare the sample. As a matter of fact, for successive experiments recorded on the same sample (adamantane) prepared in a 100 μl rotor by different operators of our laboratory, the standard deviation could be as high as 10% (data not shown).

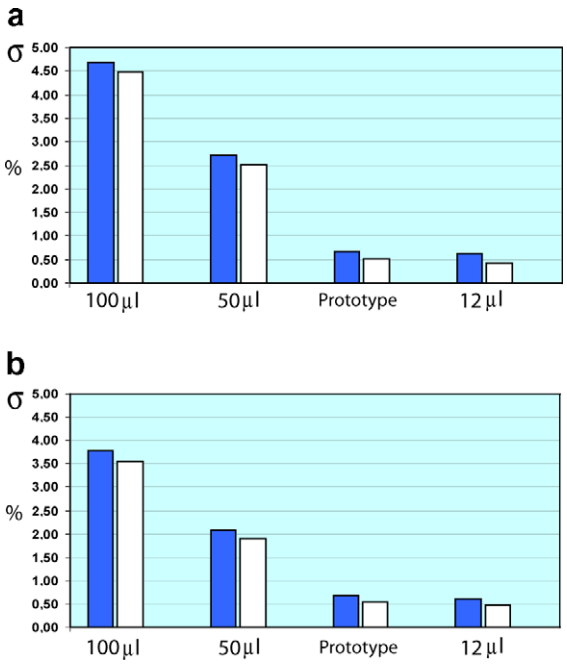

Fig. 5. Standard deviations (σ) obtained for a series of five successive SPE MAS experiments recorded at 300 K in four distinct rotors on (a) an adamantane sample and (b) a NaCl sample. For all experiments a 4 mm CP MAS probe equipped with a 12-turn detection coil was used. The white and black boxes correspond to the standard deviations obtained with and without using ERETIC™, respectively.

Finally, comparison of the standard deviations obtained on each series with and without ERETIC™ indicates that, in all cases, the use of ERETIC™ systematically reduces the standard deviation. Whatever the sample or the rotor used, this improvement seems somewhat constant and equals about 0.2 percentage point. Therefore, this gain in precision is relatively more significant for the optimized volume experiments, for which the obtained standard deviations are intrinsically lower. All these data suggest that the highest precision is achieved by using the rotor prototype together with ERETIC™.

4. Conclusion

To optimize the sensitivity and precision of quantitative ssNMR measurements, the quantitative coil volume, namely the region where the relationship between the amount of sample and the NMR response is linear, should be preliminary determined. Because of the approximate cylindrical symmetry of the detection coil, this quantitative volume can be inferred from the projection of the B_1 excitation profile along the rotor axis. Two methods are proposed to achieve this. When the probe is equipped with a magic angle gradient coil, the profile can simply be obtained by recording an image of the sample with a Hahn echo acquired in the presence of a magnetic field gradient. Otherwise, a series of NMR spectra can be recorded, while the amount of sample introduced inside the rotor is successively increased in regular amounts. Once the quantitative probe volume is known, a specifically designed rotor packing approach can be used to match the effective sample volume of the rotor to the quantitative volume of the probe. Although 4 mm rotors were compared in this work, the same trends are expected to be observed for all types of rotors. Overall, the use of this methodology allows the precision of ssNMR quantitative measurements to be drastically increased. Further enhancement in precision can be achieved by combining it with ERETIC™. Moreover, the accuracy of the analysis is expected to improve compared with non-optimized quantitative approaches. Applications for quantitative measurements based on CP MAS experiments are currently under investigation.

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