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EDGE ARTICLE

A low-cost strategy for ^{43}Ca solid-state NMR spectroscopy†

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Calcium-43 is a notoriously insensitive probe for nuclear magnetic resonance (NMR) due to its low natural abundance and small magnetic moment. Up until today, the few performed ^{43}Ca solid-state NMR studies used either highly expensive ^{43}Ca -enriched samples, or time consuming experiments on bulk natural abundance samples. We introduce the application of a magic-angle spinning coil (MACS) detector for a low-cost approach to ^{43}Ca NMR spectroscopy of solid materials with high detection sensitivity. We demonstrate 1D and 2D ^{43}Ca NMR spectra of a 1 mg ^{43}Ca -enriched sample. The sample cost is about 150 €. The effect of off-resonance MACS is also presented here.

NMR is one of the primary analytical techniques to characterize local atomic environment. For example, ^1H and ^{15}N are two of the key NMR nuclear probes for providing structural and/or dynamic information on bio-molecules.¹ Silicon, sodium and aluminium are three of the most abundant elements in the earth's crust, and thus in numerous materials. As such, their NMR observable isotopes (^{29}Si , ^{23}Na and ^{27}Al) are some of the most commonly examined nuclei in the NMR characterization of solid materials.² Calcium is also one of the abundant elements in the earth's crust, and plays key roles in various materials (glasses, bio-minerals, *etc.*), yet ^{43}Ca NMR studies are scarce. This is because ^{43}Ca solid-state NMR is intrinsically insensitive. The sole NMR observable isotope, calcium-43, is a spin-7/2 quadrupolar nucleus with an extremely low natural abundance (0.14%), and a very low Larmor frequency. One strategy employed to acquire ^{43}Ca NMR is enrichment of the ^{43}Ca isotope. However, this is a rather unpopular approach because of the extremely high cost. Regardless, ^{43}Ca -enriched 1D and 2D NMR studies have been reported.^{3–7} Natural abundance ^{43}Ca NMR was first demonstrated by Dupree *et al.* in 1997,⁸ but not until recent years, has it emerged as an alternative approach. This is joined by recent advances in NMR instrumentation and pulsed-methodology. For example, the stable MAS sample spinning of large volume MAS rotors enables an increase in the amount of sample (more than 500 mg), thus increasing the number of spins and the signal intensity. The use of the highest magnetic field spectrometers also contributes to the sensitivity gain because of the field dependency of the Boltzmann population and the quadrupole line-narrowing. Furthermore, many robust NMR-pulsed experiments are available for signal enhancement of quadrupolar nuclei.⁹ With

the advances in NMR, the inexpensive natural abundance ^{43}Ca NMR approach has been successfully applied to a wide range of solid materials (organic molecules,¹⁰ inorganic salts,¹¹ apatites,^{12–14} cements^{15,16} and ancient Egyptian pyramid¹⁷) and has illustrated the sensitivity of ^{43}Ca NMR spectroscopy to the local structural environment, leading to a common conclusion that ^{43}Ca could be a useful NMR probe for material characterization. Unfortunately, these studies require long acquisition times even for a simple 1D spectrum—more than 10 h (or even days). For this reason, correlation NMR experiments involving ^{43}Ca may not be feasible or practical using this strategy.

In this study, we introduce a low-cost and high-sensitivity approach to ^{43}Ca NMR spectroscopy on solid materials which utilizes the recent development of resonant inductively coupled magic-angle coil spinning (MACS) detection.¹⁸

The ^{43}Ca and ^1H MACS detectors used here were constructed by manual winding of micro-solenoids. Experimental coil parameters and a photo of the setup are available in electronic supplementary information (ESI†). Fig. 1 displays a single-pulse (SP) ^{43}Ca MAS spectrum of a full 4 mm MAS rotor of enriched $\text{Ca}(\text{OH})_2$ sample (~140 mg). The total cost for the sample used here is about 20,000 €. The spectrum reveals a single second-order quadrupolar lineshape corresponding to the single calcium site in the $\text{Ca}(\text{OH})_2$ structure.¹⁹ The fitted quadrupolar parameters are in agreement with the previously reported values:¹¹ a quadrupolar coupling constant of 2.57 ± 0.02 MHz and asymmetry parameter of 0.08 ± 0.02 .

On the other hand, the ^{43}Ca NMR spectrum with MACS (Fig. 1) was successfully obtained from only 1 mg of the same enriched sample. The capability of obtaining spectra from such a small quantity means the cost of the sample material is dramatically decreased; from 20,000 € to 150 €. Normally, due to the loss in the filling factor (sample-volume to MAS coil-volume ratio), a significant decrease in signal sensitivity is expected for a 1 mg sample in a 4 mm MAS probe; however, an inductively coupled ^{43}Ca MACS solenoid is used to regain the filling factor close to 1, resulting in a suitable ^{43}Ca signal after just

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† Electronic supplementary information (ESI) available: Details of MACS fabrications and its solenoid properties, NMR experimental section, 1D ^{43}Ca MQ-MACS spectrum. See DOI: 10.1039/c0sc00609b

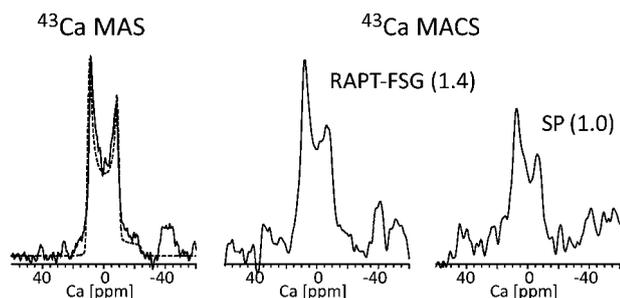


Fig. 1 Spectral comparison between ^{43}Ca MAS and MACS of 66% ^{43}Ca -enriched $\text{Ca}(\text{OH})_2$; the sample mass was 140 mg for MAS and 1 mg for MACS. The relative signal-to-noise ratios are given in parentheses. The spectral fit ^{43}Ca MAS lineshape is indicated by the dashed line. ^{43}Ca MAS was carried out with a single $\pi/2$ -pulse (SP) of 4 μs at 25 W of input power. 16 scans were collected with 30 s recycle delay (8 min). For ^{43}Ca MACS, the $\pi/2$ -pulse of 2.25 μs at 2.5 W of input power was used with a recycle delay of 30 s and 160 scans (1.3 h). All experiments were performed under sample spinning of 4000 ± 1 Hz. No ^1H -decoupling was used. RAPT-FSG pulses were performed using 10 composite Gaussian-shaped pulses, each of 89.6 μs duration operating at a B_1 of ~ 28 kHz and with offset frequencies of ± 250 kHz.

160 scans. Although accumulation of more scans could indeed enhance the signal-to-noise ratio, our wish here was to demonstrate the ability to obtain spectra in a timely fashion and the extremely-long relaxation (T_1 in minutes) for $\text{Ca}(\text{OH})_2$ imposed such a “limit”. It should be emphasized that the spectrum acquired using MACS demonstrates an excellent sensitivity compared to previous natural abundance ^{43}Ca MAS studies, including $\text{Ca}(\text{OH})_2$,¹¹ which required over 10,000 scans.

To further improve the sensitivity of MACS, the rotor-assisted population transfer (RAPT) with frequency-selective Gaussian (FSG)²⁰ pulses was applied prior the single $\pi/2$ -pulse. The use of RAPT resulted in an enhancement of the observable central-transition signal by a factor of about 1.4 compared to the standard SP spectrum. Similar enhancement factors have been previously reported for ^{43}Ca MAS with RAPT.²¹ It is noteworthy that the MACS coil endured the 896 μs long series of rapid composite Gaussian pulses without any signs of arcing or other damage to the MACS coil suggesting that NMR experiments with more complicated pulsing schemes (*i.e.* decoupling and recoupling pulses) could be implemented with MACS.

The ratio of the measured ^{43}Ca radio-frequency field produced by the MACS coil to that of the standard MAS probe coil ($B_1^{\text{MACS}}/B_1^{\text{MAS}}$), at the same input power is about 4 times higher. From the principle of reciprocity,²² this ratio indicates an enhancement in sensitivity (per unit mass) over the MAS system by a factor of 4. Thus, the experimental time with MACS is reduced by a factor of 16 for the same sample size. The signal enhancement for this ^{43}Ca MACS coil was not as good as the previously reported study (MACS in a 4 mm MAS system),¹⁸ which had a 14-fold enhancement in MACS sensitivity compared to MAS. This is due to the inevitable poorer quality factors (Q) for both MACS and MAS solenoids at low resonance frequencies (*i.e.* $Q^{\text{MACS}}(^{43}\text{Ca}) \approx 49$; $Q^{\text{MAS}}(^{43}\text{Ca}) \approx 56$). Moreover, the resonance frequency for the MACS solenoid is about 2 MHz greater than the actual ^{43}Ca on-resonance frequency, 33.58 MHz, at 11.75 T. This is attributed to the imperfection of fabricating the inductive MACS solenoid. With such a large discrepancy

($\sim 6\%$), a significantly reduced efficiency of the MACS detector is expected, leading to a reduction in the relative signal enhancement ($B_1^{\text{MACS}}/B_1^{\text{MAS}}$).²³

$$\frac{B_1^{\text{MACS}}}{B_1^{\text{MAS}}} = E \sqrt{\frac{V^{\text{MAS}} Q^{\text{MACS}}}{V^{\text{MACS}} Q^{\text{MAS}}}} \quad (1)$$

where V is the solenoid volumes; the coupling efficiency E is dependent on an inverse square of the off-resonance frequency,

$$E = \frac{1}{\sqrt{\left(1 + (k_c/k)^2\right) \left(1 + 4Q^2_{\text{MACS}}(\Delta\omega/\omega_L)^2\right)}} \quad (2)$$

where ω_L is the on-resonance frequency; the MACS off-resonance $\Delta\omega = |\omega_L - \omega_{\text{MACS}}|$; k_c and k are the critical coupling constant and coupling constant respectively, between MACS and MAS solenoids. With the same coil aspect ratio (length/diameter) for MACS and MAS, the coupling constants can be expressed as:

$$k = \sqrt{V^{\text{MACS}}/V^{\text{MAS}}} \quad (3)$$

$$k_c = \frac{1}{\sqrt{Q^{\text{MAS}} Q^{\text{MACS}}}} \quad (4)$$

On the basis of the above equations, any frequency discrepancy between the MACS solenoid and the on-resonance frequency would decrease the efficiency and diminish the signal enhancement. Fig. 2 shows the effect of MACS resonance offset using the measured quality factors found for the MACS and MAS systems. The relative signal enhancement ($B_1^{\text{MACS}}/B_1^{\text{MAS}}$) is reduced to about 62% from the optimal on-resonance condition. As a result, the ^{43}Ca MACS set-up here is not at an optimal condition, approximately a 6.5-fold enhancement could be achieved with an on-resonance MACS solenoid. On the other hand, a perfectly tuned inductive MACS solenoid might lead to difficulties with tuning the commercial MAS probe, especially when its coupling k is large. This is due to the limited tuning

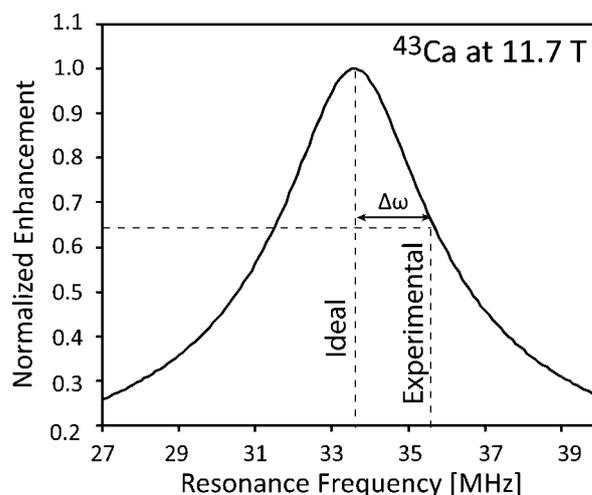


Fig. 2 A simulated plot of a relative signal enhancement ($B_1^{\text{MACS}}/B_1^{\text{MAS}}$) versus the MACS resonance frequency. The plot is based on eqn (1). The signal enhancement is normalized to its on-resonance frequency (ω_L). It shows the non-optimal enhancement for the off-resonance ($\Delta\omega$) MACS used in this study.

range of MAS probes, but it can be easily circumvented by careful changes in the probe electronics. Nonetheless, the 1D NMR results shown in Fig. 1 demonstrate that the micro-volume MACS detector could be an excellent analytical tool for precious samples, where analysis of bulk samples could be extremely costly in either monetary or in time consumption.

Recently, a 2D ^{43}Ca correlation MAS spectrum, with $\text{R}^3\text{-HMQC}$ experiment, has been reported for calcium-43 enriched apatite.⁵ The report illustrates the feasibility of the correlation experiment even for low- γ nuclei, where direct dipole-dipole couplings (^1H - ^{43}Ca) are generally small. Here, we decided to test the possibility of ^1H - ^{43}Ca correlation experiments with MACS. Inukai *et al.*²⁴ have demonstrated a double-resonance MACS experiment by fabricating a single MACS solenoid tuned at two resonant frequencies (^1H and ^{13}C). This is not an easy task especially for low- γ nuclei such as ^{43}Ca , because it requires more turns in the solenoid to achieve a low resonant frequency solenoid and thus more Cu wire (see ESI†). Moreover, the increase in conductive materials could generate significant eddy current-induced heating during rapid sample spinning.²⁵ To simplify a double-resonance experiment with MACS, we decided to use a singly-tuned MACS solenoid for ^1H detection, and complement with the standard MAS solenoid for ^{43}Ca excitation. This approach has several advantages: (1) ^1H MACS inserts are simple to fabricate and can be implemented in any MAS probe; (2) Higher signal sensitivity is achieved from inverse ^1H MACS detection; (3) Fewer number of coil turns in ^1H MACS reduces eddy current induced heat. In addition, the relaxation time for ^1H in $\text{Ca}(\text{OH})_2$ is much shorter than that for ^{43}Ca and the experiment can be repeated faster (*i.e.* 5 s recycle delay). Fig. 3 displays a 2D ^1H -detected spectrum with coherence transfer performed by cross polarization.²⁶ The 2D spectrum reveals a standard ^1H spectrum along the direct dimension (horizontal axis) and a ^{43}Ca MAS spectrum along the indirect dimension (vertical axis). A correlation signal is clearly visible between the OH proton and the single calcium site in the 2D spectrum. Although the sensitivity is rather low because of the small number of ^{43}Ca nuclei in the 1 mg sample, and the weak $\text{OH}\cdots\text{Ca}$ dipolar coupling (~ 318 Hz), the singly-tuned ^1H MACS approach for double-resonance illustrates the potential for multiple resonance experiments with MACS. Further details regarding such double-resonance MACS developments can be found in ref. 27.

High-resolution ^{43}Ca MQ-MAS was also attempted with MACS as a preliminary test for multiple-quantum experiments with insensitive nuclei. Unfortunately, the long recycle delay (30 s) renders the acquisition of a complete 2D 3Q-MAS spectrum impractical. Only a 1D spectrum of the first t_1 -increment of the 3Q-MAS experiment was acquired (see ESI†). Nonetheless, the observed first t_1 signal indicates that the ^{43}Ca MQ-MAS experiment is possible for micro-sized samples with MACS; especially for samples with fast relaxation. For example, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and CaHPO_4 could be good candidates because of their rather short relaxation times.¹¹ Moreover, additional enhancement schemes²⁸ and/or the exploitation of high radio-frequency fields generated with micro-coil²⁹ could further enhance the sensitivity for the high-order coherence (3Q or possibly even 5Q).

NMR hardware manufacturers have recently introduced MAS probes equipped with smaller rotors (1.2–1.8 mm o.d.) capable of

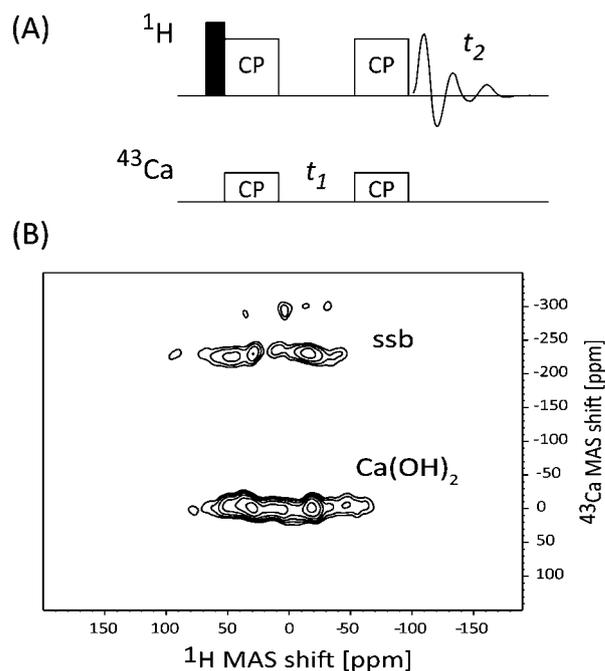


Fig. 3 (A) Pulse sequence for a 2D ^1H -detected cross-polarization experiment. (B) The 2D CP spectrum was recorded with a singly-tuned ^1H MACS coil for optimal ^1H (inverse) detection, and ^{43}Ca -excitation was obtained using the standard coil of the MAS probe. A ^1H $\pi/2$ -pulse of $3.25\ \mu\text{s}$ with 10 W of input power was used. The CP experiment was performed under $\nu_{\text{rf}}(^1\text{H}) \sim 72\ \text{kHz}$ and $\nu_{\text{rf}}(^{43}\text{Ca}) \sim 4\ \text{kHz}$ with 5 ms contact periods. The 2D spectrum was recorded with States phase-cycling, 128 t_1 increments, 480 scans, and a recycle delay of 5 s. The spectrum displays a Ca-H correlation signal for $\text{Ca}(\text{OH})_2$, and its spinning-sideband (ssb).

acquiring good-quality spectra from a few microlitres ($\sim 2\text{--}9\ \mu\text{L}$) of sample. These probes are specially designed for MAS experiments with high-frequency nuclei and do not have much capability with those of low-frequency nuclei. Thus, modification of the MAS probe (*e.g.*, increased number turns in coil and/or addition of capacitance) is necessary. Furthermore, the signal sensitivity per unit mass is still not as good as MACS because the filling factor for the small MAS probe is not as large as the MACS design. In addition, the incorporation of MACS in MAS experiments is rather straightforward, and the solenoid design is flexible and simple, thus in principle, it is possible to implement MACS into the current advancements of cryogenic MAS.³⁰

This study has demonstrated a highly sensitive and low-cost approach, to ^{43}Ca solid-state NMR. A good example where this low-cost NMR approach could be useful is the application to titration studies with NMR spectroscopy, where analysis of various samples with different elemental contents is carried out. Recent multinuclear NMR studies,^{13,14} including natural abundance ^{43}Ca NMR, was applied to investigate the cationic substitution in hydroxyapatite structures by measuring multiple hydroxyapatite samples with different content of ions. Although natural abundance samples are inexpensive, long acquisition times (2 days) were needed to acquire each of the ^{43}Ca NMR spectra. The high-sensitivity ^{43}Ca MACS demonstrated here would be a good and low-cost option for such studies. For some calcium-containing compounds, synthesizing 1 mg ^{43}Ca -enriched

sample may not be a trivial task because of the difficulty in handling such a small quantity. However, producing only a few milligrams would make the synthesis procedure easier, and is still less costly than the quantity required for a standard solid-state NMR analysis. Similar applications with MACS could readily be performed to other low- γ nuclei (e.g., ^{17}O , ^{33}S , ^{73}Ge , ^{25}Mg , ^{87}Sr , etc.) and/or other high valued samples. Furthermore, this approach could be proven invaluable in studies of radioactive compounds where even if the synthesis must be performed in large quantities, one would prefer to minimize the quantities needed for chemical analysis and characterization. The availability of high radio-frequency²⁹ from a micro-coil is also beneficial for low- γ nuclei with expected wide-line features such as ^{67}Zn in a metalloproteinase.³¹ The singly-tuned inverse-detection MACS approach to double-resonance experiments could also be a key component to widening this NMR methodology to magnetic resonance microscopy. Furthermore spinning resonators such as MACS could offer new possibilities for DNP-MAS in ultra-high magnetic fields which is a direction we are currently exploring.

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