Application Note 22

Hyperpolarisation in NMR using *para*-hydrogen

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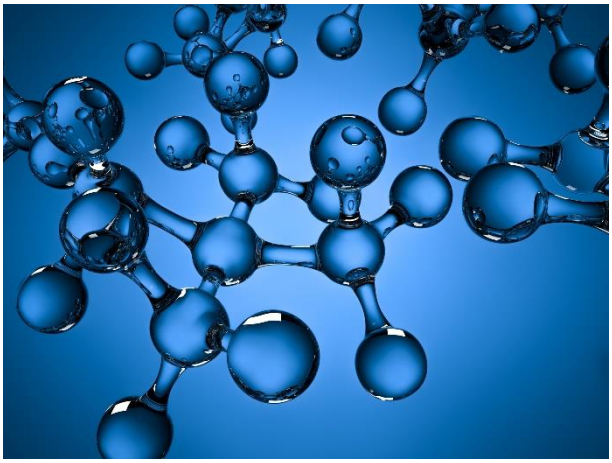
Introduction

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful and flexible methods of analysis used in science today. However, in comparison with other analytical techniques, NMR signals have inherently low sensitivity. This is because the strength of the NMR signal is dependent on the overall nuclear magnetisation; and when magnetised/polarised by an external magnetic field, only a small proportion of a bulk sample contributes towards the overall magnetisation. Which, for ^{1}H, effectively means that only one in every 10,000 – 100,000 nuclei are observed (depending on the magnetic field strength), with the ratios even less favourable for other, low natural abundance, low gyromagnetic ratio nuclei, such as ^{13}C, ^{29}Si & ^{15}N.

In order to overcome this low sensitivity in NMR, a wide range of approaches can be used, together known as hyperpolarisation. These use a range of methods that feature chemical manipulations, photochemistry and NMR pulse sequences; to introduce additional polarisation, and hence increase the intensity of the observed signal, usually by two to four orders of magnitude.[*Chem. Rev.*, 2023, **123**, 1417-1551]

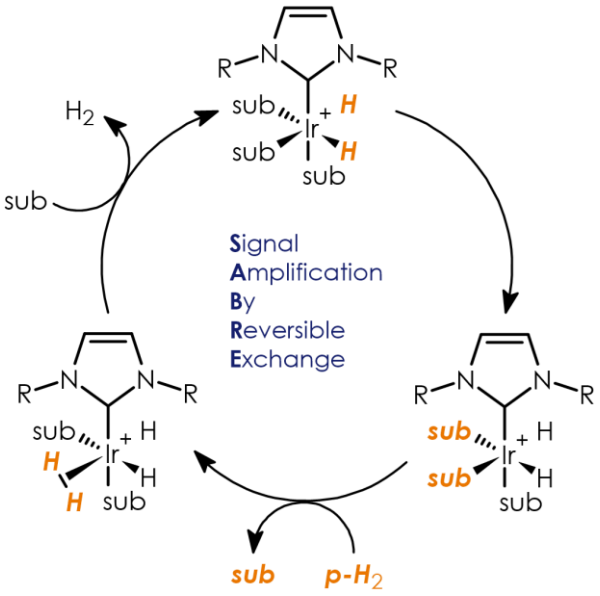
In this application note, we show some preliminary data to demonstrate how easily hyperpolarised NMR spectra for a range of nuclei can be obtained on the **Oxford Instruments X-Pulse Broadband NMR Spectrometer**, using the SABRE method to perform the hyperpolarisation without the need for extensive time consuming optimisation.



SABRE

Signal **a**mplification **b**y **r**eversible **e**xchange (SABRE) was pioneered by Simon Duckett and his team at the University of York, and uses *para*-H_2 (a spin isomer of H_2, which can be easily obtained by cooling H_2 to cryogenic temperatures) to transfer polarisation *via* an iridium complex, to a substrate which is then observed by NMR (*Scheme 1*). Compared with many other methods for hyperpolarisation, SABRE is relatively straightforward, requiring only a source of *para*-H_2,

To perform the SABRE method for hyperpolarisation, *para*-H_2 is added to a sample containing an iridium pre-catalyst, target substrate, and any co-reagents. These samples are then shaken in a magnetic field to transfer the polarisation from the *para*-H_2 to the substrate, and a one-dimensional NMR spectrum obtained, the resulting phase of the hyperpolarised signals depend upon the exact experimental conditions used.[*Annu. Rep. NMR Spectrosc.* 2018, **93**, 145-212]



Scheme 1 Generic mechanism for **S**ignal **A**mplification **B**y **R**eversible **E**xchange (SABRE)

Hydrogen-1 (Proton)

A ^{1}H NMR spectrum of a *ca* 60 mmol/ℓ solution of methyl-4,6-d_2-nicotinate following hyperpolarisation by SABRE is shown in *Figure 1*. The two aromatic protons are clearly hyperpolarised, and observed as singlets with negative phase; while the methyl ester signal retains a positive phase without significant enhancement. The two aromatic signals (each corresponding with a single proton), are 150× more intense than the methyl ester signal (corresponding to three protons).

Another feature to note in the spectrum are the signals at δ_H −14.0 & −18.2 ppm, these are hyperpolarised signals from an iridium hydride complex, formed as the pre-catalyst is converted to the active catalyst (pictured in *Figure 1*); therefore, showing there's sufficient signal enhancement to allow for observation of reaction intermediates on the **X-Pulse**.

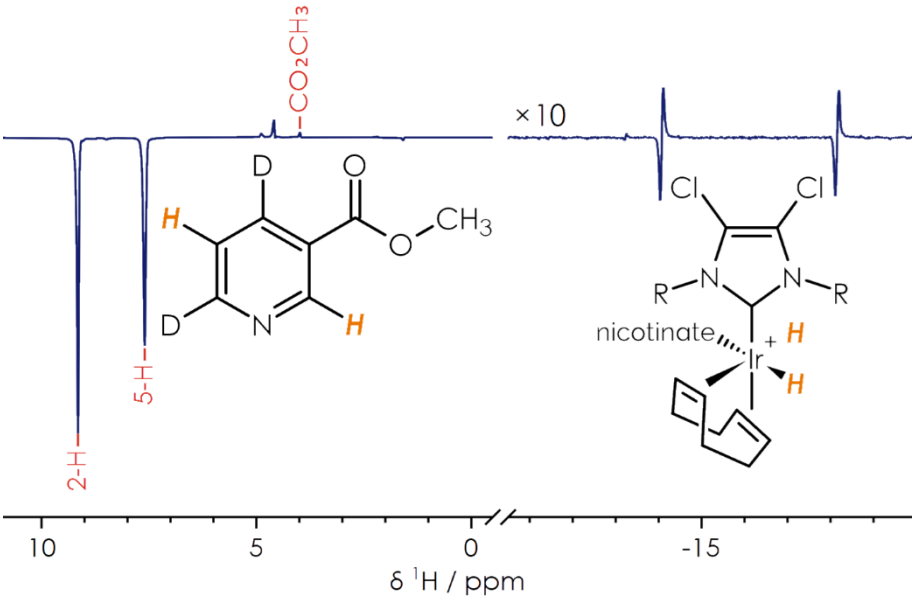


Figure 1 Hyperpolarised ^{1}H NMR spectra (single scan) of methyl-4,6-d_2-nicotinate in the prescence of an iridium dihydride intermediate

Fluorine-19

Like ^{1}H, ^{19}F NMR spectra can display significant enhancement with hyperpolarisation. In *Figure 2* a single scan spectrum of a *ca* 60 mmol/ℓ solution of 3-fluoropyridine, following hyperpolarisation by SABRE is shown. The signal-to-noise ratio (SNR) of this signal is over one thousand. While for the same sample without hyperpolarisation; the signal can still be observed in a single scan, but has an SNR of approximatly two (see *Figure 2* insert).

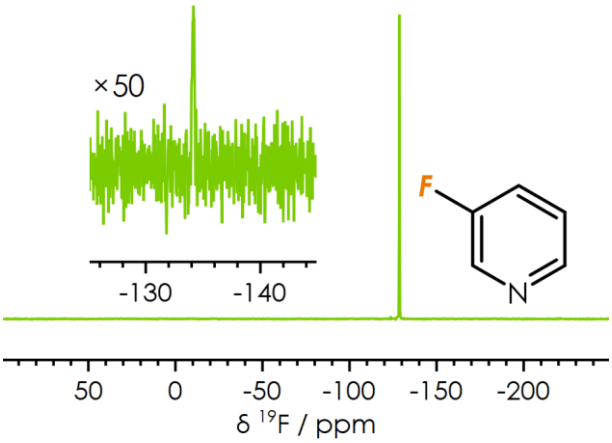


Figure 2 Hyperpolarised ^{19}F NMR spectrum (single scan) of 3-fluoropyridine (Insert: single scan spectrum without hyperpolarisation)

Carbon-13

Despite how important carbon-13 NMR spectroscopy is for organic, organometallic, and inorganic chemistry; it's amongst the lowest receptivity nucleus commonly studied by NMR. Therefore, ^{13}C NMR benefits greatly from methods to increase the signal intensity, such as hyperpolarisation and isotopic labelling. Both of these methods are applied in this example.

A $^{13}C\{^{1}H\}$ NMR spectrum was observed in a single scan following hyperpolarisation by SABRE of *ca* 30 mmol/ℓ sodium 1,2-pyruvate-[$^{13}C_2$] (*Figure 3*); with the polarisation transfer taking place in a mG field (lower than the strength of the Earths magnetic field), a method known as SABRE-SHEATH.[*Angew. Chem., Int. Ed.*, 2019, **58**, 10271-10275]

There are two doublets clearly visible (with opposite phases) in the spectrum, with 63 Hz, ^{13}C-^{13}C *J*-coupling. These peaks have SNRs as high as 1700; while without hyperpolarisation, even with this level of enrichment, at benchtop frequencies the sample is too dilute to observe any signal with just a single scan.

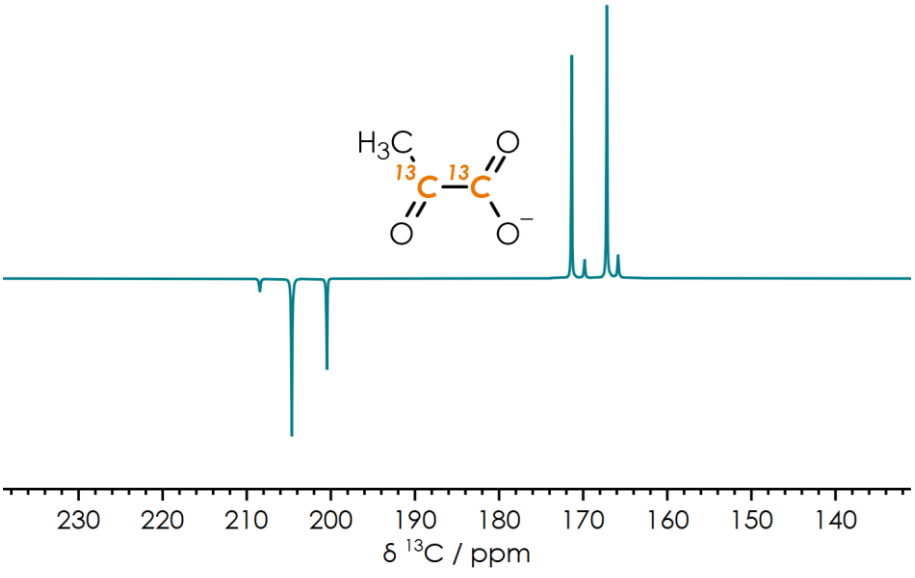


Figure 3 Hyperpolarised $^{13}C\{^{1}H\}$ NMR spectrum (single scan) of sodium 1,2-pyruvate-[$^{13}C_2$]

Silicon-29

Due to both low natural abundance and a low gyromagnetic ratio, silicon-29 is amongst the lowest receptivity nuclei observed by NMR. With even 1 mol/ℓ samples often taking over an hour (>1000 scans) to observe a signal in a standard one-dimensional NMR spectrum at benchtop frequencies. Indeed, polarization transfer sequences (INEPT & DEPT) are routinely used for ^{29}Si NMR due to the signal enhancement they give.

To demonstrate the signal enhancement possible using SABRE, a sample containing *ca* 30 mmol/ℓ tris(*iso*-propyl)silanol, the iridium catalyst, and benzylamine was exposed to *para*-H_2, and a ^{29}Si{^{1}H} NMR spectrum obtained where a signal is clearly observed in a single scan (*Figure 4*). In this two step mechanism, polarisation is initially transferred from *para*-H_2 to the benzylamine *via* the iridium complex; then from the benzylamine to the silanol by exchange of labile protons; with the process therefore known as SABRE-Relay.[*Angew. Chem., Int. Ed.*, 2020, **59**, 2710-2714] To directly observe this ^{29}Si signal, for a compound at this concentration, at benchtop frequency, without hyperpolarisation, would take many hours or even days.

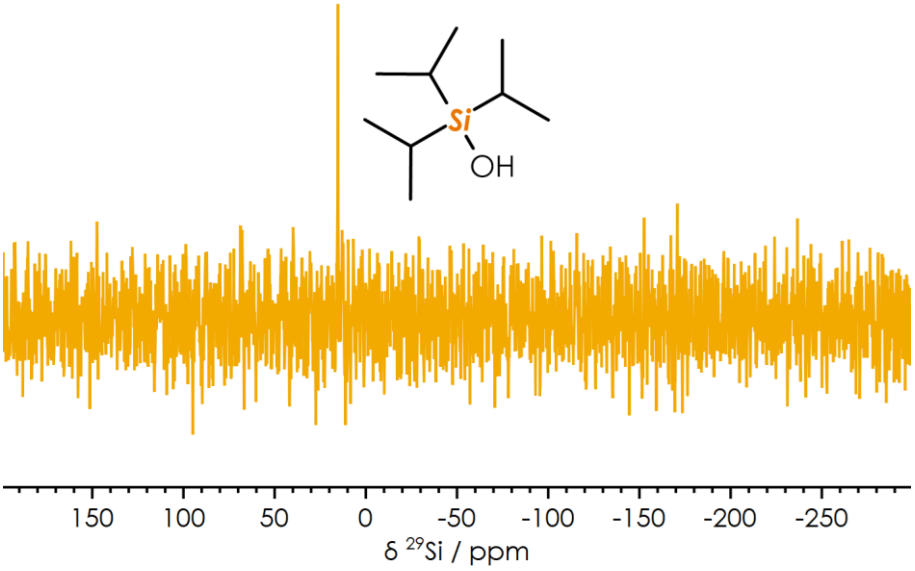


Figure 4 Hyperpolarised ^{29}Si{^{1}H} NMR spectrum (single scan) of tris(*iso*-propyl)silanol

Summary

Hyperpolarisation using SABRE has been demonstrated for a range of nuclei on the **Oxford Instruments X-Pulse Broadband Benchtop NMR Spectrometer**. These are only some of the many nuclei which can be observed on a *single* X-Pulse.

The X-Pulse uses a 1.4 T (60 MHz) cryogen-free permanent magnet, and comes with a fully tuneable user removable probe with an *external* deuterium lock as standard. Along with an optional 25 position autosampler, optional variable temperature capability, and an optional flow cell. Allowing for maximium flexibility to perform a range of experiments, including many different methods of hyperpolarisation.

If you have any questions about this application note, please contact our experts: magres@oxinst.com

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