HyperSense®

DNP for NMR

HyperSense®

Delivering Sensitivity to NMR

The Business of Science™

OXFORD INSTRUMENTS

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Delivering Sensitivity to NMR

HyperSense - the *in-vitro* DNP Polariser

HyperSense allows the user to drive NMR sensitivity to levels never seen before. The sensitivity improvements attainable with ^{13}C, ^{15}N and ^{29}Si have the potential to increase the breadth of your Nuclear Magnetic Resonance (NMR) experiments and your applications.

Examples are:

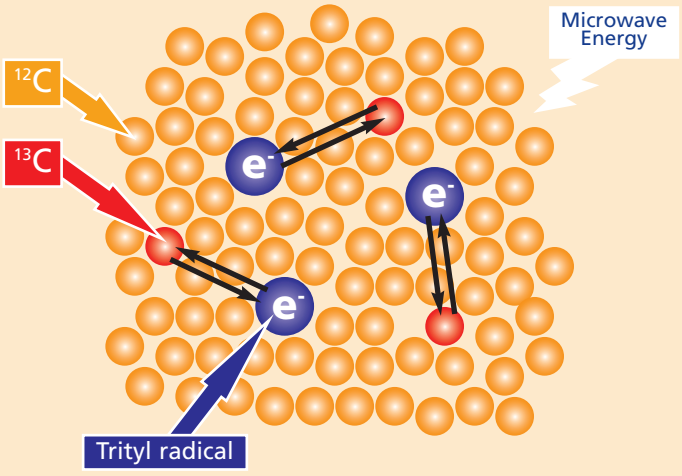
- Shorter data collection times and/or the use of much smaller amounts of sample material.
- Benefits for quaternary carbons that will improve structure confirmation and elucidation. This could provide more reliable NMR spectral assignments in a shorter time.
- The ability using direct detection of ^{13}C to identify complex biomixtures in metabonomics.
- Using the enhanced signal to carry out chemical kinetic experiments.

HyperSense, an instrument that configures easily with your laboratory and existing spectrometer.

What is DNP?

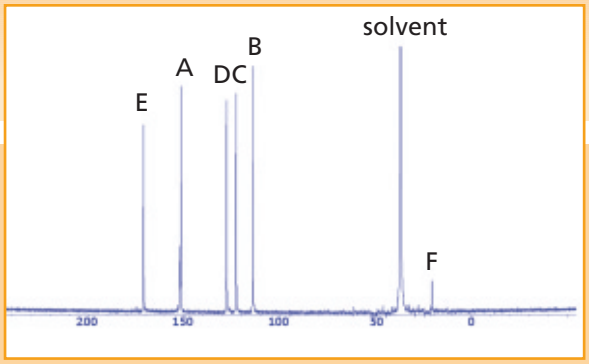
Dynamic Nuclear Polarisation (DNP) is the method for obtaining strongly polarised nuclear spins in solution, thereby delivering sensitivity enhancement for your application.

In a glassy state the free electron of the radical, at low temperature, in the presence of a magnetic field can transfer the polarisation to nearby nuclei using microwave irradiation.



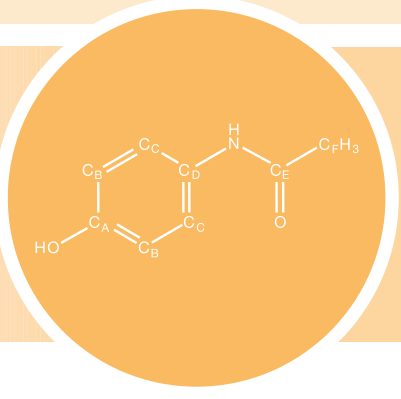
Enhanced ^{13}C Spectral Content

- After polarisation, carbon nuclei are enhanced. This makes detection quicker compared to conventional NMR.



4 hours polarisation, single scan
Limit of detection S/N of 10 on the smallest peak F

^{13}C-DNP of acetaminophen natural abundance, 2µmol (300µg)



Working with **HyperSense®** in your Laboratory

1. Preparing your sample for DNP

Dissolve your sample in a glassing agent (e.g. DMSO/H_2O). Add proprietary radical (typically 15mM). Place sample in polariser sample cup (10 - 200µl). Load sample into polariser.

2. Sample Conditioning

Sample in polarising region. Sample cooled to ≥1.4K

3. Polarising

Irradiate unpaired electrons with microwaves (over 15 minutes to 4 hours) to transfer polarisation

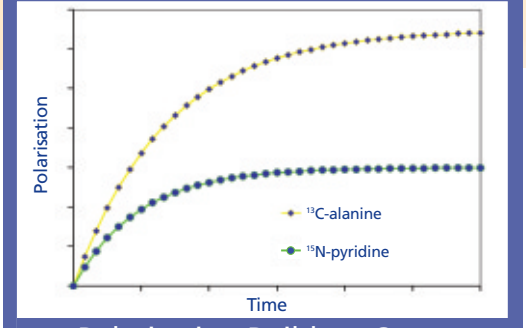
4. Transfer

Sample dissolution using hot solvent. Polarised sample is transferred

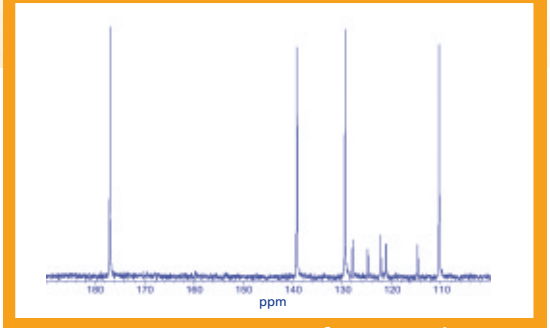
5. Acquisition

HyperSense triggers NMR acquisition

The Automated Process of Polarisation



Polarisation Build-up Curve



^{13}C DNP spectrum of tryptophan (selected region)

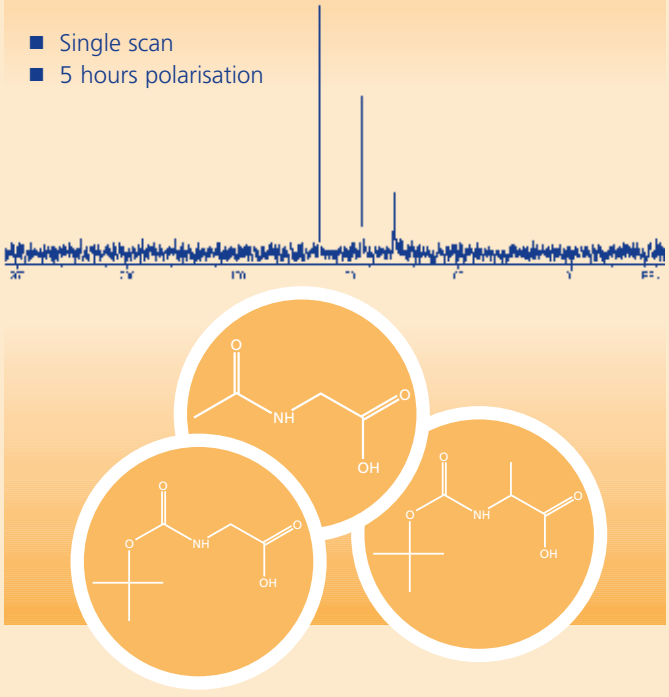
What does **HyperSense®** deliver?

Examples of challenging direct detection

^{15}N Direct Detection

^{15}N of mixture of L-alanine t-BOC, glycine t-BOC and N-acetyl glycine (10mg of each)

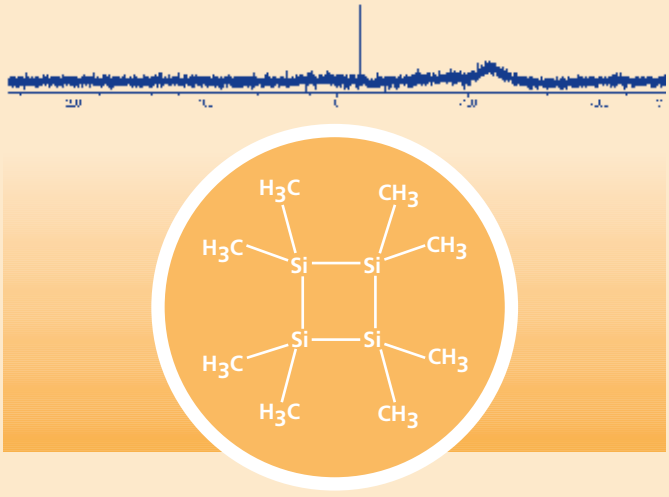
- Single scan
- 5 hours polarisation



^{29}Si Direct Detection

^{29}Si of Volasil-244 (9.5mg)

- Single scan
- 5 hours polarisation



Features of HyperSense:

High levels of sample polarisation

- Enables the user to directly detect ^{13}C, ^{15}N and ^{29}Si more quickly than with conventional NMR
- Requires smaller amounts of sample, down to µg level

After polarisation some carbons are particularly enhanced

- Enhanced spectral content improving peak assignment

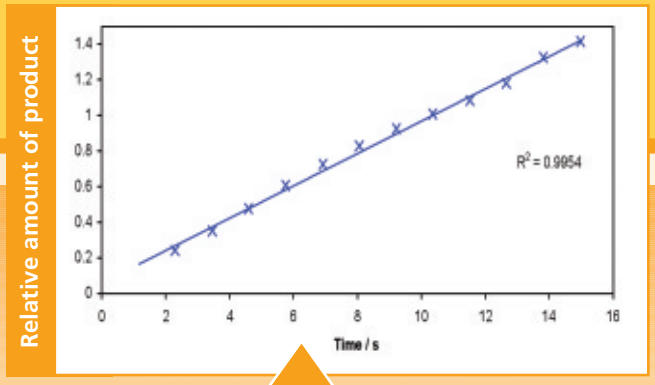
Separate hyperpolarisation module

- Enables dual functionality of conventional NMR and DNP-assisted spectroscopy

Case Studies using **HyperSense®**

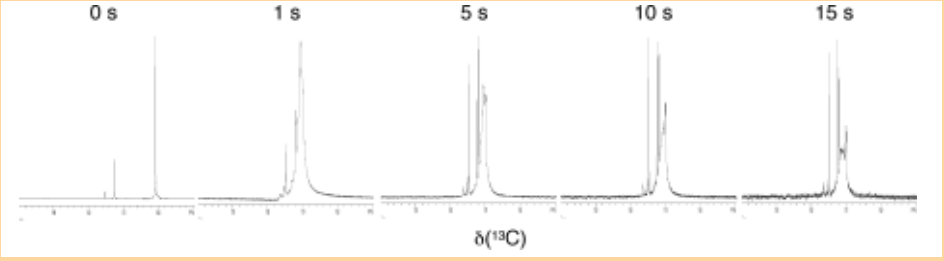
1. Chemical Kinetics

Low sensitivity limits kinetic studies using NMR. Long acquisition times restrict most studies to ^{1}H. For the first time HyperSense provides real time multiple ^{13}C spectra from a single sample. Direct ^{13}C detection improves spectral resolution and makes the observation of carbon reaction centres possible.



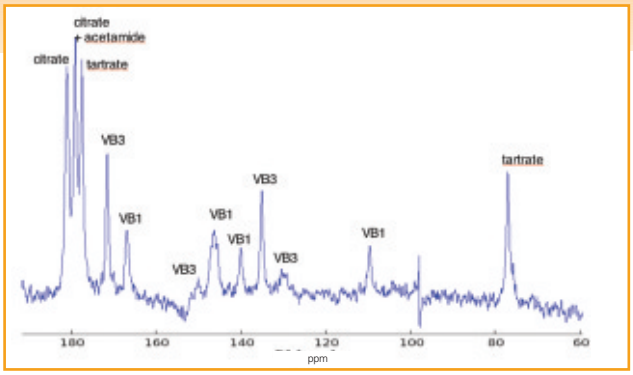
From ratio of peak integral, at 172.3ppm (reaction:control) kinetic rates can be determined[1]

$^{13}C_1$ Ethylpyruvate + NaOH $\xrightarrow{MeOH}$ methylpyruvate + pyruvic acid + further components



- $^{13}C_1$ Ethylpyruvate polarised for 100min
- Sample transferred using methanol to NMR magnet
- 5mm NMR tube contains 75mM NaOH solution
- ^{13}C spectra acquired one per second

2. Detection of biologically relevant molecules in mixtures

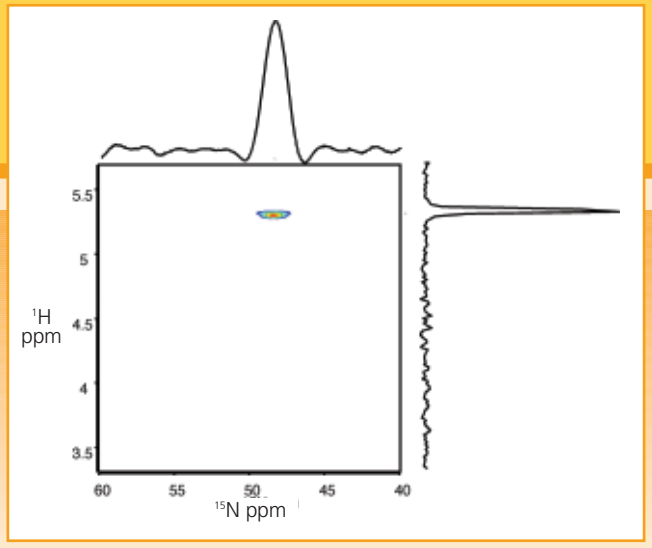


NMR of complex mixtures can be difficult due to low sensitivity and spectral crowding for ^{1}H spectra. Conventional ^{13}C NMR offers greater chemical shift dispersion but with lower sensitivity.

The single-scan ^{13}C spectrum of a compound mixture (citrate, acetamide, tartrate, vitamins B1 and B3) shows the work being carried out at Birmingham University into DNP NMR of complex mixtures[2]. Using HyperSense the NMR spectrum of multiple molecules can be enhanced, so aiding interpretation.

Case Studies of **HyperSense®**

3. Enhanced 2D NMR



2D NMR provides information rich correlated spectra.The single enhanced spectrum produced by HyperSense can be used in conjunction with the SNR requirements of the ultrafast 2D single scan method developed by Professor Lucio Frydman[3,4].

The figure shows a ^{1}H-^{15}N HSQC spectrum of ^{15}N labelled urea, 130µM. Natural abundance materials at low concentration can also be used to record both ^{1}H-^{13}C and ^{1}H-^{15}N HSQC spectra[5].

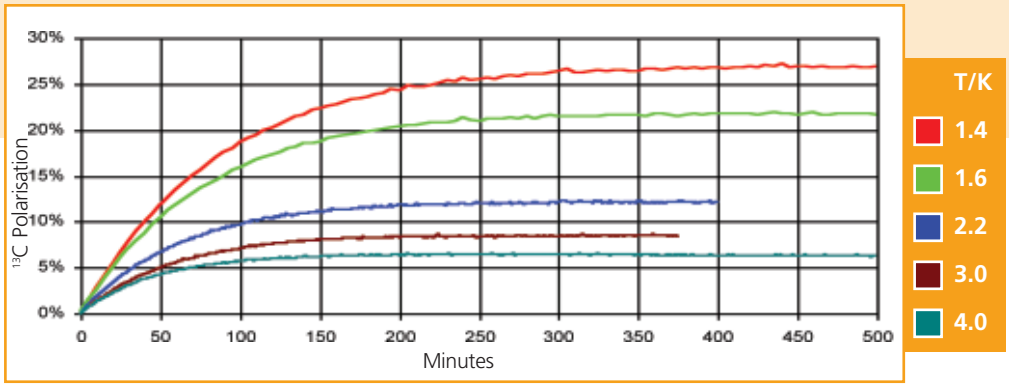
4. Research into DNP

DNP is a new and exciting area of NMR spectroscopy which in itself provides huge scope for research.

Sample polarisation is affected by: temperature; microwave power; radical identity and concentration; polarisation time and the nature of the sample.

By way of example the figure below shows the effect of temperature. With increasing temperature there is an inverse dependence on polarisation and the rate of polarisation build-up.

HyperSense offers the user the ability to change the polarisation parameters[6].



Other applications include:

- Structure verification
- Impurity identification
- Ligand screening
- Biomarker discovery

Summary

HyperSense will transform your research using ^{13}C, ^{15}N and ^{29}Si. It will enable you to surpass previous sensitivity barriers and increase the applicability of NMR spectroscopy by:

- **Increasing signal to noise ratio up to 10,000 times[7]**
- **Reducing experimental runtimes for ^{13}C and ^{15}N applications**
- **Dramatically lowering limit of signal detection**
- **Switching rapidly and simply between NMR and DNP NMR**

Let us work with you to show you the benefits of DNP NMR with HyperSense. At Oxford Instruments we have several demonstration facilities across the world staffed with expert scientists to enable you to assess the benefits of DNP NMR to your applications.

Performance Specification and Services

System performance

Nuclei available:	^{13}C, ^{15}N, ^{29}Si and other spin $^{1}/_{2}$ nuclei
Sample polarisation times:	Typically 15 minutes to 6 hours
NMR spectrometer compatibility:	All current spectrometer platforms
NMR RF probe requirements:	Operation with 5 and 10mm probes
Dissolution solvents:	Water and methanol
Polarising agent:	'Trityl radical' typical usage 0.2-5mg per sample

Instrument specification

Microwave source:	Integrated 94GHz with 0.5GHz sweep, (user selectable) up to 100mW
Magnetic field strength:	3.35T actively shielded
Sample temperature:	Selectable <3.9K, ≥1.4K[8]
Helium refill volume:	65L from minimum level
Nitrogen refill volume:	60L from minimum level
Helium consumption during dissolutions:	Typical <2L per dissolution[9]
Minimum operating ceiling height:	<3.0m
System weight including cryogens:	600kg approx
Trigger output for NMR spectrometer:	5V signal

Services

Compressed air/nitrogen:	6 bar min
Helium gas:	6 bar min, grade 99.999% pure from cylinder
Exhaust line:	Vacuum exhaust line or suitable safety compliant filter
Electrical:	Europe: 230V, 50Hz, 2.5A single phase and 415V, 50Hz 20A three phase USA: 110V, 60Hz, 3A single phase and 208V, 60Hz, 20A three phase Japan: 100V, 50-60Hz, 3A single phase and 200V, 60Hz, 20A three phase

Dimensions

Footprint:	W 1668mm (~66") x D 1038mm (~41")
Height:	2860mm (~113")

click onto **www.oxford-instruments.com** for more information

References and notes
(1) Detection of Minor Isomers and Study of Chemical Kinetics by DNP NMR – Presented at SMASH 2006, Damir Blazina, Steven Reynolds, Oxford Instruments Molecular Biotools Ltd., Oxon, UK.*
(2) Probing DNP Enhancement for Biological Samples - Presented at ENC 2006, Christian Ludwig[1], Abdul Hamid-Emwas[1], Damir Blazina[2], Steven Reynolds[2], Andrew Sowerby[2], Ulrich Günter[1]. [1]CR UK Institute for Cancer Studies, University of Birmingham, Birmingham, UK, [2]Oxford Instruments Molecular Biotools Ltd., Oxon, UK.*
(3) Frydman, L.; Scherf, T.; Lupulescu, A. *Proc. Nat. Acad. Sci.* **2002**, *99*, 15858.
(4) Using the 2D single scan technology requires a license from the Weizmann Institute of Science.
(5) *Ultrafast Two-Dimensional Nuclear Magnetic Resonance Spectroscopy of Hyperpolarised Solutions*, L. Frydman and D. Blazina, Nature Physics, in press.
(6) Characterising Solid-State DNP, Rob Slade, Graham Hutton, Damir Blazina.*
(7) Ardenkjaer-Larsen, J.H.; Fridlung, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M. H.; Servin, R.; Thaning, M.; Golman, K. *Proc. Nat. Acad. Sci.* **2003**, *100*, 10158.
(8) As measured by instrument control system with 65% helium insert level at microwave power 100mW over 30 minutes.
(9) Averaged over 10 repeatable runs with a temperature of 1.4K over a 6 hour polarisation and dissolution, 100mW microwave power with helium level at 65%.
*Please see www.oxford-instruments.com.

Ref: Hyp/0307

HyperSense is not marketed for *in-vivo* use.

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