Whitepaper

From R&D to QC, making NMR accessible for everyone.

Putting NMR spectroscopy at the heart of the analytical chemistry lab

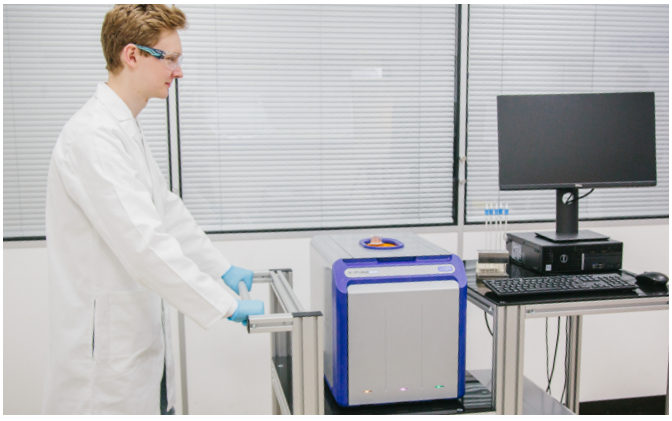
William G. Hale [1], James T. Sagar [1], Rosie E. Jones [1], Robin J. Blagg [1]

[1] Oxford Instruments Magnetic Resonance, Halifax Road, High Wycombe, Buckinghamshire, HP12 3SE, U.K.

Introduction

For many decades, access to nuclear magnetic resonance (NMR) spectroscopy has been an intrinsic requirement for any analytical chemistry lab. Due to its ability to elucidate molecular structure and track reaction dynamics, this technique is recognised throughout industry and academia as a powerful, non-destructive, non-invasive method, and is taught in undergraduate chemistry curricula. But how can NMR be employed on the bench in almost any laboratory environment?

Access to NMR has often been difficult, with the requirement for substantial space, special facilities and expert users, limiting the usability of the technique in synthetic chemistry and industrial labs.

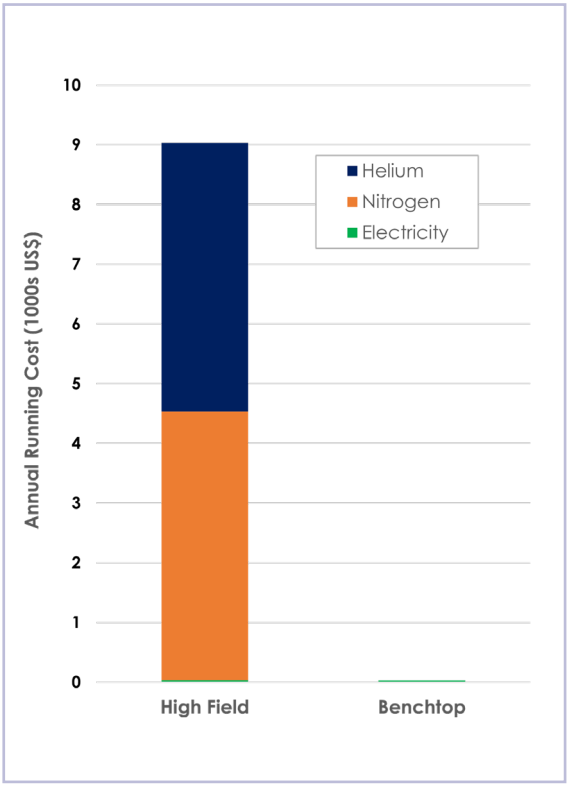


However, recent advances in benchtop NMR instrumentation, brings this technique out of the basement or centrally managed facility, and into the heart of any laboratory. Benchtop NMR instruments are a smaller, cryogen-free version of traditional high field instruments, and they even enable data collection in a fume hood. Oxford Instruments X-Pulse adds broadband capability to benchtop NMR.

This capability allows operators to use just one benchtop instrument and rapidly tune between most NMR active chemical nuclei.

This enables instrument sharing across many applications, future-proofs R&D capabilities and significantly expands the range of challenges solved across education, academia, and industry.

For industry, the benefits of small footprint, mobile, benchtop NMR are very clear: shorter preparation times, automation capabilities, fewer staff specialists, and a reduced space demand to name a few. This means time efficiencies and cost savings versus high field NMR instruments, which incur significant initial capital investment and often require dedicated rooms to operate in. Benchtop NMR fits right alongside existing instruments in a lab or can be quickly transported on a trolley between labs. As benchtop NMR is cryogen-free, it is much cheaper to run than traditional high field instruments. Our analysis showed that one can conservatively save over 9,000 US$ per year in running cost just from the cryogens alone. See graph below.



Any business looking to invest in high field NMR needs to answer these questions:

- Can one establish a reliable and continuous supply of liquid helium and liquid nitrogen?
- How will they be stored safely on site?
- Is there someone on site that is qualified to handle them in a safe manner?

As well as the on-going, and ever rising cost of cryogens, there are also the peripheral logistics of buying and installing a high field instrument to consider.

Research and Development

While early applications largely focused on basic one-dimensional ^{1}H NMR spectra, modern spectrometers offer many additional capabilities beyond structural elucidation. One important example is the ability to measure self-diffusion coefficients, to extract physical information about a sample including:

- molecular size
- viscosity
- ionic conductivity and transference (e.g., in lithium-ion battery electrolytes)

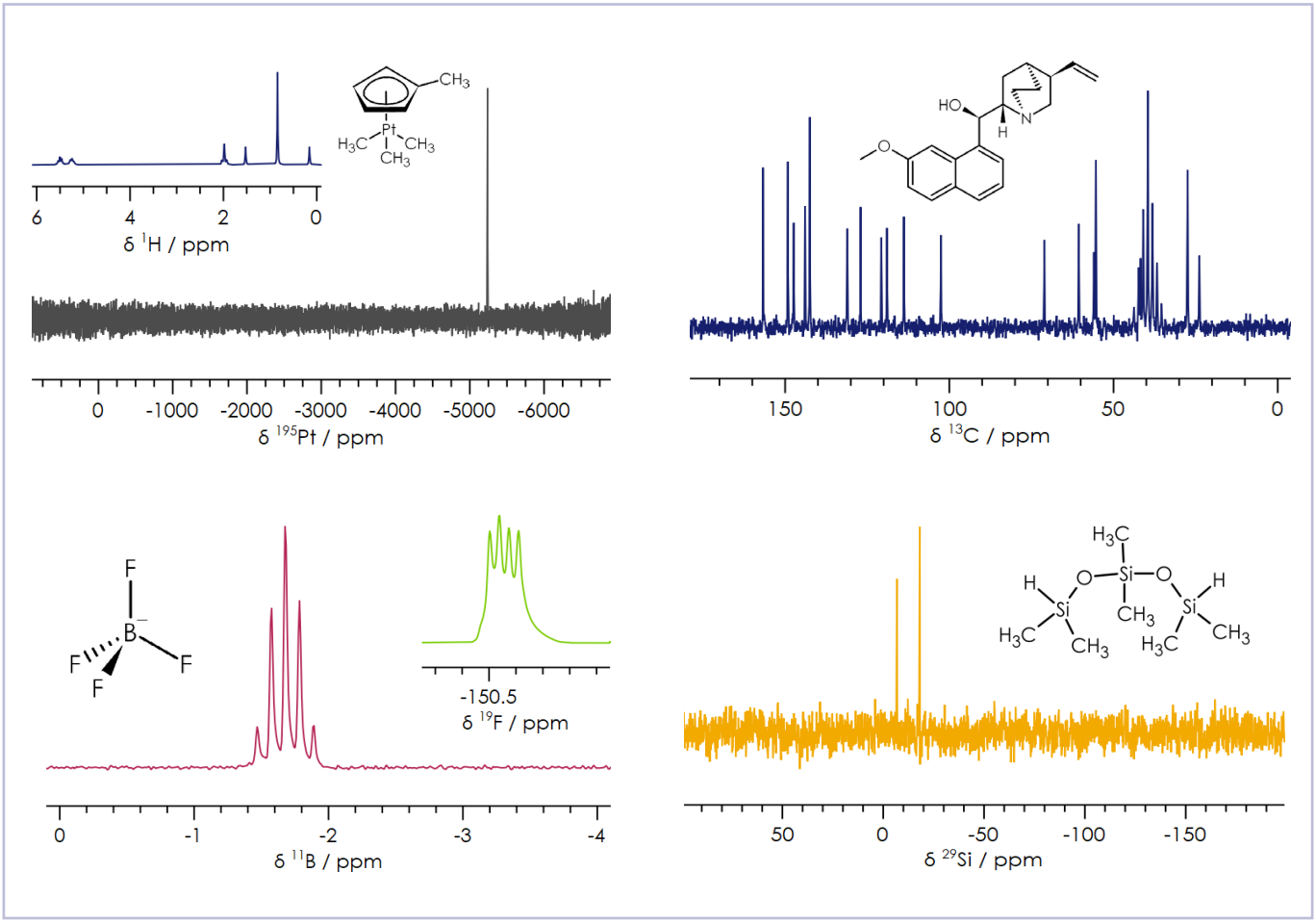


Figure 1: A sample of the various X-nuclei that it's possible to detect on X-Pulse

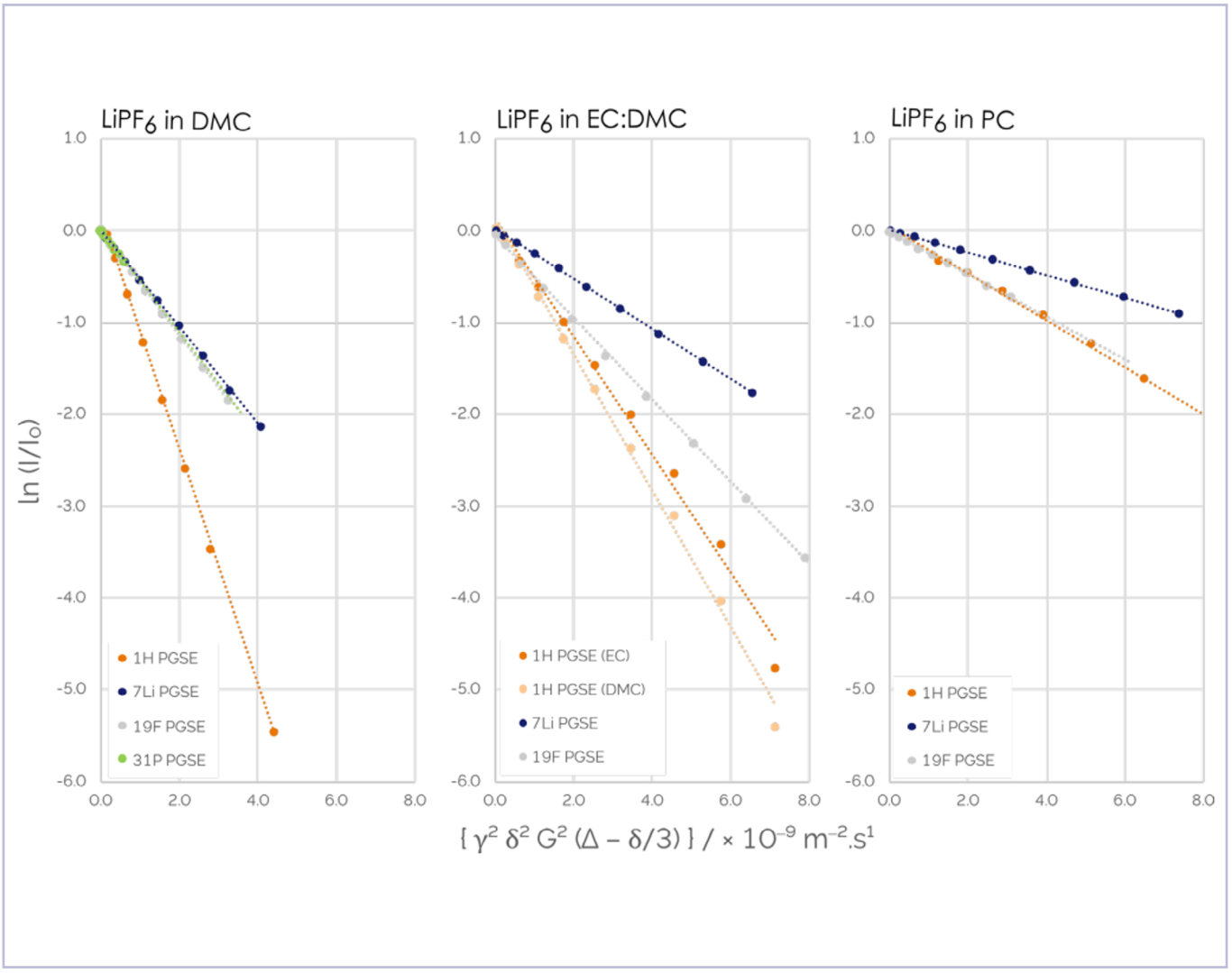
A benchtop NMR spectrometer equipped with pulsed field gradient (PFG) hardware can use techniques such as the PFG spin echo (PFGSE) experiment to determine diffusion coefficients of sample components by measuring change in NMR signal as a function of the PFG strength. Adding variable temperature capability allows the study of sample thermal behaviour under a range of expected working conditions for the samples.

Pulsed field gradients are applied to the sample and vary in intensity. As the gradient strength increases, the signal is attenuated due to the changing phase difference between molecules after the first and second gradient pulses. The attenuation can be related to the diffusion constant using the Stejskal-Tanner equation[1].

This method can be used particularly in battery research and development where measuring the self-diffusion, cationic transference, and ionic conductivity of the various lithium salts in different electrolytes can quantify performance and aid the design.

As an example, lithium hexafluorophosphate, Li[PF_6] was studied in three different electrolyte solvents: dimethyl carbonate (DMC); a 50:50 mixture of ethylene carbonate (EC) and DMC; and propylene carbonate (PC). The PFGSE spectra are shown in Figure 2. This reveals the differences in diffusion behaviour among components of a single electrolyte solution, as well as differences among the same species in different solvent conditions. Because ^{31}P is part of the same [PF_6]– ion as ^{19}F, the two should diffuse at the same rate.

Figure 2: Stejskal-Tanner plots of integrals obtained from PFGSE data of $LiPF_6$ in DMC, 50:50 EC:DMC, and PC. ^{1}H data is shown in dark orange and light orange ^{7}Li data is shown in blue, ^{19}F in grey, and ^{31}P in green. All data were acquired on a single X-Pulse broadband benchtop NMR spectrometer.



In fact, the diffusion behaviours were identical within the precision of the method. However, as expected, the diffusion behaviour of the smaller Li+ ion differed significantly from that of the larger $[PF_6]$– ions. In addition, the markedly different quantitative results in Table 1 for the same Li+ and $[PF_6]$– ions in the three solvent systems, demonstrate the importance of solvent choice in battery design. Conductivity differed by approximately a factor of three between electrolytes using PC and DMC solvents, while cation transference changed far less. Moreover, the difference in diffusion behaviour for DMC as a pure solvent, compared to the 50:50 mixture with EC, demonstrates the effects of environment on solvent molecule. By utilising the portability of benchtop NMR, these electrolyte characterisations can happen in the lab, accelerating and enhancing development capabilities from the start.

Sample	D_+, cation	D_-, cation	D, solvent	Conductivity	Transference (t_+)
	$X10^{-10}m^2s^{-1}$ @39.5c			$X10^{-3}Sm^{-1}$	
DMC	5.16	5.68 (^{19}F) 5.81(^{31}P)	12.7	3.91	0.48
EC:DMC	2.74	4.45	6.42 (EC) 7.38 (DMC)	2.54	0.38
PC	1.24	2.30	2.56	1.28	0.35

Table 1: Parameters measured by NMR for three electrolyte systems: $LiPF_6$ in DMC, in 50:50 EC:DMC, and in PC.

QA/QC

As well as providing a research and development tool, benchtop NMR offers powerful quality assurance/quality control (QA/QC) properties. One important example is in the food industry where NMR can identify adulterants or impurities in a spectrum. One industry that greatly benefits from this is coffee. Coffee is one of the most widely traded commodities in the world. The trade is made up of two main varieties, commonly known as arabica (*C. arabica*) and robusta (*C. canephora*), with arabica accounting for 60-70% of the world market. Arabica is generally considered to be of higher quality and sells on world commodity markets for about twice the price of robusta. There is therefore the potential for economic fraud, with unscrupulous traders adding robusta to arabica and still labelling the product "100% Arabica". Analytical methods are needed to detect the presence of robusta coffee in products labelled as arabica. The official method for testing for the presence of robusta in arabica is DIN 10779, which is a complex HPLC method that takes over 7 hours and involves the use of acetonitrile (a known carcinogen).

Defernez *et al* have shown that low-field (benchtop) NMR can be used instead. These researchers found that 16-O-methylcafestol (16-OMC) could be detected providing a faster, more sustainable method to detect adulteration of arabica by robusta.

A surveillance study of retail purchased "100% Arabica" coffees using this method found that 6 out of 60 samples displayed signals commensurate with adulteration at levels of 3–30% w/w[2]. 16-OMC has several clear peaks in the NMR spectrum, but one in particular, at 3.16 ppm, is in a region where there are no interfering peaks from other compounds present in the samples. Figure 3 illustrates where this peak appears in relation to spectra of various coffee samples. The peak at 3.16 ppm is labelled (i) in the figure.

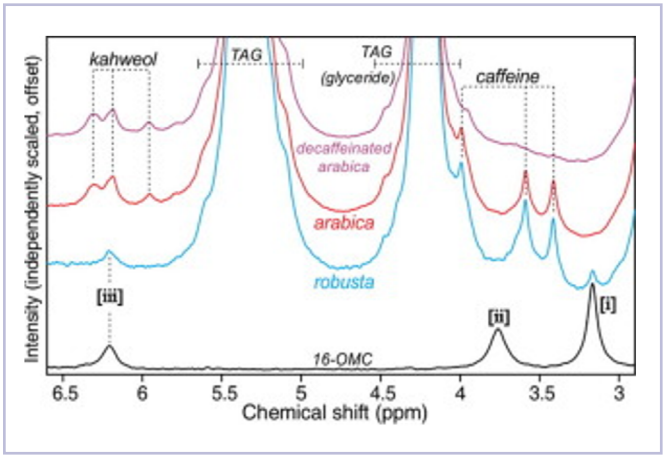


Figure 3: NMR spectra of 16-OMC and various coffee samples from Derfernez *et al*[2].

Although the trace amounts vary from sample to sample, they are generally equivalent to an addition of approximately 1% of robusta into arabica, so this is the lower limit of detection of adulteration.

The complete statistical analysis generated indicates that arabica coffees adulterated with robusta at the 1% w/w level will be detected in about half of all cases. This rises to 90% at the 2% level, and at the 3% level it is unlikely that any adulterated sample will pass undetected.

The developed method has a total preparation and measurement time of 90 minutes per sample, which is over 4 times faster than the HPLC method. By bringing NMR out of the custom-built laboratory and on to the benchtop QA/QC turnaround times are greatly improved.

Education

Synthetic chemistry education benefits from early hands-on NMR exposure to deepen understanding of the analytical technique as well as delivering structural information for the synthesised molecules and compounds. Having benchtop NMR in the lab speeds up data acquisition, so students can analyse what they have synthesised, just minutes after making it. To prepare students for a future in chemistry it is essential that they are well versed in NMR, which is employed widely throughout the chemical and biotechnology research industries.

For education, NMR can now be a much more hands on experience. To learn structural elucidation, often students are handed paper copies of spectra that have been at least partially processed for them, sometimes with challenging-to-read axes. They perform the analysis with the knowledge that all the information they require to determine the structure has been provided to them. This process is not a reality in a research environment where researchers interact with the data electronically, and where they may need to identify and run more complex spectra to complete their analysis. Using benchtop NMR, even these more complex spectra are readily available in the lab. Two-dimensional experiments are now commonplace in most instruments and the most used ones such as COSY, TOCSY, and HSQC come as standard on the X-Pulse. The extra dimension of data provided by these experiments accelerates structure determination of small molecules. You can read more about structure elucidation using X-Pulse here.

Furthermore, if samples do require higher magnetic fields, then benchtop NMR becomes an invaluable pre-screening tool to determine if the product or sample you're investigating really does warrant the time and money required for high field evaluation. This pre-screening functionality can also be useful to NMR facility managers. As well as taking up much less space than a traditional high field set-up, benchtop NMR can also free up the more expensive-to-run magnets to be used in a more efficient way.

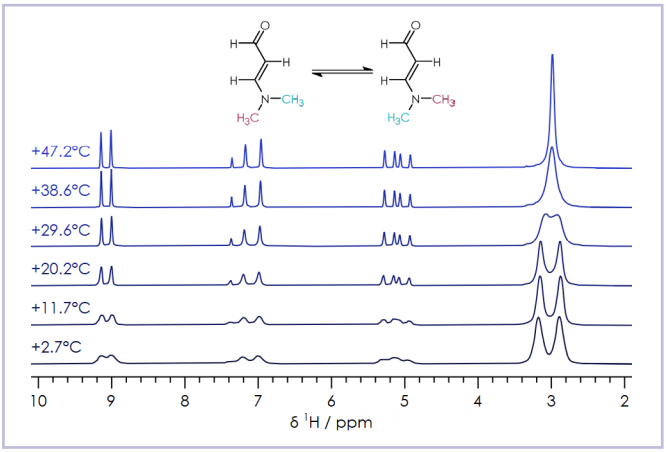


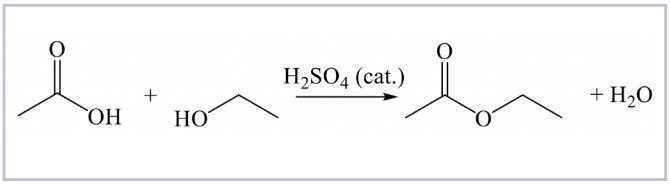
Figure 4: ^{1}H NMR spectra of 3-Dimethylaminoacrolein in $CDCl_3$, over a temperature range of +2 to +48°C.

Using the benchtop NMR variable temperature accessory, students can watch the progression of dynamic changes in molecular structure using NMR to reinforce learning concepts from both molecular structure and NMR. In Figure 4, the cis-trans isomerisation of the amino-methyl groups in 3-dimethylaminoacrolein is tracked. The NMR spectra show that as the sample group signals coalesce into a single signal. At the molecular level, increasing the sample temperature increases the rotational energy of the molecule, which speeds up the rotation of the methyl groups until they are spinning so fast that they become equivalent. From this, the thermodynamic barrier for rotation of the carbon-nitrogen bond can be calculated. With a benchtop instrument, NMR experiments can now follow a similar pattern with prediction, experiment, and result happening in the same session.

Reaction Monitoring

The ability to easily integrate flow cells into benchtop NMR also extends the benefits for both research and teaching beyond structural elucidation. With benchtop NMR, analysis can be run in minutes to determine the success of a reaction – no more running down to the user facility or sending samples to external companies for routine analysis.

One simple example is the flow rate and temperature dependent esterification of ethanol and ethanoic acid catalysed by sulphuric acid (reaction shown below). The product synthesised, ethyl ethanoate, a solvent that is used for varnishes, lacquers, dry cleaning, stains, fats, and nitrocellulose.



To investigate the rate constant dependence on temperature, a flow cell set-up (Fig 6) was used with X-Pulse and the reaction performed at varying temperatures.

Temperature /°C	Temperature / K	Rate constant / $mol^{-1}\ dm^3\ s^{-1}$
22	295	$2.3x10^{-5}$
37	310	$5.1x10^{-5}$
50	323	$1.0x10^{-4}$
57	330	$1.7x10^{-4}$
72	345	$3.6x10^{-4}$

Table 2 shows the temperature dependent variation in rate constant. NMR additionally enables determination of enthalpy (ΔH), entropy (ΔS) (associated with reaching the transition state from the reactants, and the Gibbs Free Energy (ΔG). For more information on how this was calculated please see our app note here.

Conclusion

The availability of cryogen free, benchtop NMR has enabled analytical scientists, university teaching courses, and industrial research facilities to bring the NMR technique right into the heart of their labs. Benchtop NMR has removed the high upfront and maintenance cost of traditional high-field NMR, and with the arrival of the true broadband X-Pulse NMR incorporating comprehensive flow and temperature control, a single instrument now addresses needs ranging from student teaching, right through to high end R&D and industrial QA/QC.

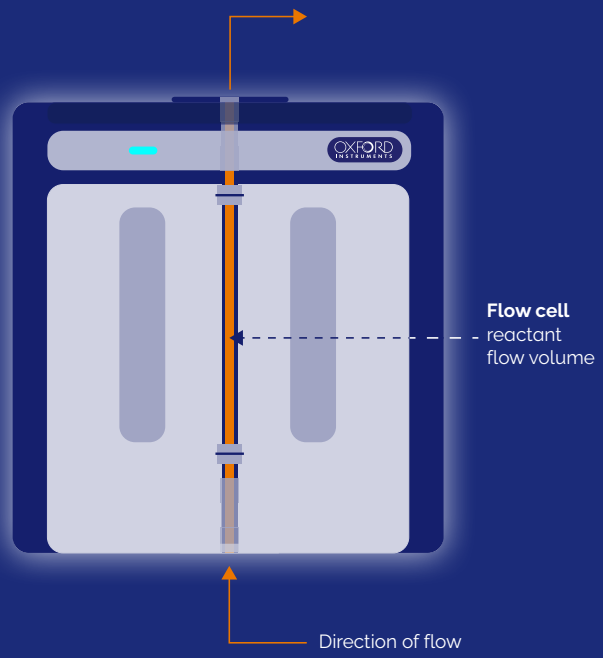
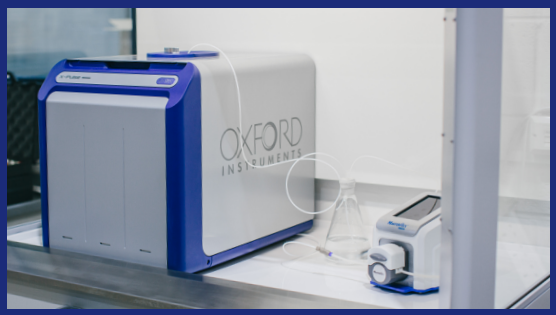


Figure 6: Schematic (above) and physical set-up (below) in a fume hood showing how flow chemistry is incorporated in the X-Pulse benchtop NMR instrument.



References

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2. M. Defernez; E. Wren; A. D. Watson; Y. Gunning; I. J. Colquhoun; G. L. Gall; D. Williamson & E. K. Kemsley, *Food Chem.*, **2017**, *216*, 106-113

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