INTRODUCTION

NMR spectroscopy underpins research across the physical and life sciences. Though historically associated with chemistry and described as a means of structural elucidation, NMR now finds application across a range of diverse topics including the characterisation of alcoholic beverages,[1] the investigation of dynamics in the solid state,[2] and the study of carbohydrates formed in the marine environment.[3] Magnetic Resonance Imaging[4] has opened up an entirely new field of study, ensuring that the subject is known to the general public, even though they may not realise it! An understanding and appreciation of NMR, how it works, and what information it provides is a fundamental tool with which the graduating chemist (and, increasingly, also scientists from other disciplines) must be equipped. However, NMR is perceived as challenging by many students, while for those teaching it, new methods and approaches are welcome, for use in both the lecture theatre and the laboratory.

A significant development within the subject over recent years has been the advent of the so-called “Benchtop NMR” spectrometer, natural successor to the relaxometer that has traditionally been utilised to carry out NMR spectroscopy at relatively low frequency. Relaxometers generally operate at ca. 0.5 T and generate relaxation data by fitting the FID, but they are very limited in application and are of little benefit in teaching. Continuous Wave (CW) spectrometers, often with electromagnets that produced stable fields up to about 2 T, were in use until relatively recently. The new generation of “benchtop” instruments are based on permanent magnets operating at 45–80 MHz (1–2 T) and, as a consequence of the high homogeneity of the field, can boast very acceptable linewidths of ca. 1 Hz for 1H NMR spectra. Despite the relatively recent arrival of this new resource, there is already a body of published work that exemplifies its versatility.[5–7] From a practical perspective, the removal of the need for cryogen refills ensures more straightforward maintenance and reduces running costs, whereas the low footprint of the cabinet leads to portability with the attraction to house the spectrometer in spaces out-of-bounds to high-resolution instruments. In addition, no significant stray field exists outside of the spectrometer and so hazards associated with exposure to static magnetic fields are no longer a concern. Many of the currently available models also accept NMR samples made in the conventional manner, that is, by dissolving a solid or liquid in a deuterated solvent and the solution transferred to an NMR sample tube. This becomes an important factor in...
undergraduate teaching, as the importance of high-quality samples is another aspect of the subject that learners often find difficult to appreciate.

Despite all of these advantages, it is important to note that the benchtops possess limitations. The low fields born by the spectrometers inevitably lead to lower resolution and sensitivity. The latter requires that samples be more concentrated, whereas the former increases the likelihood of signal overlap and the risk of distortions caused by second-order effects, that is, the departure from idealised appearance of multiplets caused by the similarity in value of J-coupling constants and chemical shift difference between the coupled spins. These aspects of the instrument’s operation do necessitate careful consideration of the types of systems to which the benchtop NMR might be applied. Clearly, confusion will result if students are receiving lectures on signal multiplicity arising from J-coupling in model systems and they then observe nonideal distorted patterns when they acquire their own spectra. A firm understanding of the expected multiplicity splittings in well-behaved examples must precede the discovery of higher-order spectra. Notwithstanding these issues, however, there is great scope for the use of the benchtop NMR in undergraduate teaching. A number of papers have already highlighted applications in this area, such as identifying the products of the radical bromination of ethylbenzene \[8\] and determining the \( T_1 \) and \( T_2 \) relaxation time constants of protons in caffeine \[9\].

The use of high-resolution NMR spectrometers is now routine and largely automated. Even complex experiments can be carried out at the touch of a button. Whilst making it easier to obtain spectra, this path has led to a “mystification” of NMR, removing much of the interaction between student and machine, which has inspired previous generations of chemists to become NMR spectroscopists. Benchtops are designed to be easy to use but do offer the opportunity to introduce users to various functions that would be kept fixed by integrated systems, for example, adjusting the number of scans, spectral width, transmitter offset, line broadening, number of data points, and more. All of these parameters can be easily edited on the benchtop NMR, and students can observe for themselves what the consequences are for their spectra.

In this letter, we address a selected range of concepts that we feel can be difficult to grasp for undergraduate students studying NMR spectroscopy whilst being amenable to clarification either with the sole aid of the “benchtop” or in some cases the benchtop in combination with a high-resolution instrument. These ideas are illustrated by examples from our own work.

## 2 | EXPERIMENTAL

Samples of 1,2-dichlorobenzene were prepared in CDCl\(_3\) (0.7 mL). Ethylbenzene samples were standard calibration samples supplied by Bruker, at 0.1% and 10% by volume in CDCl\(_3\) (0.7 mL). The whisky sample was prepared as a 50% by volume solution of the spirit in D\(_2\)O (0.7 mL).

NMR experiments were carried out on a Nanalysis NMReady 60e 60-MHz spectrometer equipped with a proton-observe probe and a Bruker AVIIIHD 400 MHz spectrometer equipped with a BBF(O) broadband probe. For the 1,2-dichlorobenzene experiments on the 60-MHz spectrometer, the relaxation delay was set at 2 s, number of scans 16, and TD = 8,192. For the experiments on the 400-MHz spectrometer, the relaxation delay was set at 1 s, number of scans 16, and TD = 32 K. For the ethylbenzene and whisky samples on the 60-MHz spectrometer, the relaxation delay was set at 2 s, number of scans varied according to the protocol described in the discussion section, and TD = 2,048.

Processing of all NMR data was carried out using standard Bruker TopSpin® packages, either TS 2.1.5 or TS 3.6.0 or Spinworks 4, version 4.2.3.0.\[10\]

## 3 | RESULTS AND DISCUSSION

### 3.1 | Influence of magnetic field strength on resolution

The frequency (\( \nu \)) of NMR signals is dependent on the magnitude of the externally applied field (\( B_0 \));

\[
\nu = \frac{g \beta_N B_0}{\hbar},
\]

where \( g \) is a number specific to each isotope, \( \beta_N \) is the value of the nuclear magneton, and \( \hbar \) is Planck’s constant. Frequency therefore scales linearly with \( B_0 \). The concept of the chemical shift,

\[
\delta = \frac{\nu_{\text{sample}} - \nu_{\text{ref}}}{\nu_{\text{ref}}},
\]

which is field-independent can be daunting to the uninitiated and especially so the notion that a single unit of chemical shift represents a different frequency range at different magnetic field strengths. As a consequence of this, NMR spectra may look different when recorded on spectrometers with magnets of different sizes.

The \(^1\)H NMR spectrum of ortho-dichlorobenzene presents a classical example for an AA’XX’ (AA’BB’) spin
system that will always remain higher-order no matter how high the NMR spectrometer frequency might be. The spectrum itself consists of two half-spectra, each containing up to 10 (12) lines and a characteristic symmetry (Figure 1). The two half-spectra are mirror images to each other and display noticeable roofing effects. Direct analysis of the spectrum is possible as it is ultimately characterised by just four coupling constants and two chemical shifts. The AA$^0_{0XX}$ system of ortho-dichlorobenzene is readily recognised at high field. However, what is not often appreciated by students is that the appearance of the NMR spectrum will change completely on a benchtop NMR and do so in a predictable fashion.

Students readily appreciate that $H_A$ and $H_{A'}$ (and similarly $H_X$ and $H_{X'}$) will have the same chemical shift but often struggle to understand the origin of the higher-order effect, which arises because the coupling constant between $H_A$ and $H_X$ is not the same as between $H_A$ and $H_{X'}$, and the coupling constant between $H_X$ and $H_{X'}$ is not zero.

At 9.4 T (400 MHz), it is possible to analyse the spin system and deduce all coupling constants and chemical shifts. A full analysis of this system, based on the work of Günther, is included in the Supporting Information. The calculated values correlated well with those reported in the literature. As a final check, the simulated spectrum based on these chemical shifts and coupling constants is in excellent agreement with the experimentally observed spectrum.

The appearance of the $^1$H NMR spectrum of ortho-dichlorobenzene changes completely when going from high field to 60 MHz, and this can be nicely illustrated with the help of a benchtop NMR spectrometer. Although it is no longer possible to analyse the spin system unambiguously at low field, the 60-MHz NMR spectrum can still be simulated using the parameters determined at high field (Figure 2).

This experiment demonstrates the concept of chemical shift well, the values for this parameter being essentially unchanged at the two different fields, 9 T versus 1.4 T. However, the frequencies do change according to the relationship between $\nu$ and $B_0$ and move closer together at 1.4 T whereas the coupling constants remain, as their name implies, constant.

### 3.2 Sensitivity and signal-to-noise

A complementary issue, and similarly troubling to undergraduates, is the notion of sensitivity. Like resolution, sensitivity is field-dependent, and the generally acceptable quality parameter, the signal-to-noise ratio ($S/N$), scales with $B_0^{3/2}$. $S/N$ is also proportional to the square root of the number of scans in the acquisition. The concepts of resolution and sensitivity are easily confused, and because of the frequent use of automated NMR experiments where the spectral parameters are pre-defined, it is difficult to demystify these. Benchtop spectrometers, with their user-friendly interfaces, enable the operator to alter the values of key spectral parameters on the fly and therefore gain insight into the effects that these modified entries have on the final spectrum. Mistakes are to be encouraged, and the robust nature of the instrument ensures minimal opportunities to inflict damage.

One approach is to record spectra of standard samples on the same spectrometer using varying numbers of scans and to note the consequences for the quality of the spectrum. A simple illustration how the quality of a spectrum improves with increasing number of scans is best visualised by recording a sample of low concentration. Figure 3 shows a series of spectra acquired from a dilute

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**FIGURE 1** $^1$H NMR spectrum of ortho-dichlorobenzene acquired at 9.4 T (400 MHz) showing the characteristic AA$^0_{0XX}$ splitting pattern.

**FIGURE 2** $^1$H NMR spectrum of ortho-dichlorobenzene acquired at 1.4 T (60 MHz), from a 30% solution in CDCl$_3$. 
sample of 0.1% ethylbenzene in deuterated chloroform, utilising 1, 16, and 64 scans. After 1 scan only the aromatic signal is just about recognisable, whereas after 64 scans, all resonances are apparent and the multiplets arising from the CH₃ and CH₂ groups discernible. It is also clear from these spectra that the “amount of improvement" on moving from 16 scans to 64 scans is less pronounced than when moving from 1 to 16 scans, indicating that, for very dilute samples, running very long accumulations will not result in ever-improving spectra.

### 3.3 Mixtures and quantitation

Students are often under the impression that NMR spectroscopy is a technique exclusively devoted to the analysis and characterisation of pure compounds. To help dispel this myth, it is important to get chemistry undergraduate students to practise analysing simple mixtures. An ethanol–water mixture, which could be an alcoholic beverage, has just about the right level of difficulty for a beginner to tackle. NMR allows the relative amounts of components to be readily determined in such a mixture. Some data analysis and processing is also involved, extending the learning outcomes and complementing teaching in more traditional areas of analytical chemistry. Finally, the NMR analysis of alcoholic beverages provides an opportunity to introduce NMR spectroscopy to the non-scientist, for example at an Open Day.

The concept of alcohol-by-volume (ABV), the percentage of alcohol present (by volume) in beer or wine, will be familiar to many. The alcohol content of beers, wines, and spirits is measured traditionally with a hydrometer, essentially a weighted bob that will sink to a degree in the liquid depending on the density. As sugars are converted to alcohol during the brewing process (fermentation), the density changes, and the difference in density before and after fermentation provides an estimate for the alcohol content.

Figure 4 shows the 1.4 T spectrum of a 50% solution of a Single Malt Scotch Whisky in D₂O. The only signals visible are those of ethanol and water. Several groups have reported approaches to determine ABV with the help of a benchtop NMR.[13–15] An attractive way of translating this idea is to ask students to construct a calibration curve from a series of prepared mixtures of ethanol and water of known relative concentrations and then to use this curve to determine the ABV values of real alcoholic beverages. The calculations are straightforward and will assist student understanding of the use of integration to obtain the composition of a mixture. An example of a calculation is included in the supplementary information.

A calibration curve was produced from a series of 14 standard samples of ethanol–water mixtures (Figure 4, inset). Each point is derived from an average of three integral values, each standard sample having been run in triplicate. In the Supporting Information, we show one of the three spectra acquired for the calculation of each point.

A number of commercial samples were tested in this way (Table S2). Again, each sample was run in triplicate and the calculated ABV averaged. In most cases, the values obtained are close to those advertised, but there are variations. In some instances, this may be due to the real ABV of the beverage not matching that advertised.
but this is unlikely to be the case for all of the entries. In addition, there are errors associated with the calculations; we estimate up to 5%. The largest errors arise for samples of low alcohol concentrations where the integration is less accurate and at high concentrations where the plot deviates from linearity. It should also be noted that the integrations for the CH₂ protons can be affected by the presence of signals from sugars (e.g., in liqueurs) that occur in the same region of the spectrum. The results obtained are critically dependent on the rigour of the sample preparation and the accuracy of the processing of NMR data. Optimal baseline correction, phasing, and integration of the latter are crucially important and so this experiment provides ample opportunity for students to practise these skills. Relatively inexpensive hand-held metres are available that will provide ABV values of alcoholic beverages through density measurements, and students may therefore be able to check the numbers produced by NMR against those generated by a more traditional method. Ultimately, the principle of the process is the most valuable aspect, and any errors or inconsistencies that arise can be used serve as discussion points to further enlighten student understanding of NMR spectroscopy.

4 | CONCLUSIONS

This brief survey of simple experiments amenable to “benchtop” NMR spectroscopy will promote a number of key concepts: resolution and the effects of varying the external Bo field on chemical shift dispersion, sensitivity, J-coupling, and the quantitative analysis of mixtures. Despite its limitations, a benchtop NMR offers a number of significant advantages over conventional high-field hardware in a teaching lab. Accessibility for students, ease of use, and the ability to change parameters on-the-fly and to make mistakes without causing major disruption contribute to make the NMR spectrometer less of a black box.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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