

A Hands-on Problem-Based NMR Experiment using a Benchtop Spectrometer for the Undergraduate Laboratory

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Abstract A newly-designed problem-based laboratory experiment utilizing a benchtop NMR spectrometer was implemented for undergraduate students. The aim of the experiment was to have a hands-on, problem-based approach that focused on structure elucidation as well as practical aspects such as sample preparation, data acquisition and manipulation. The experimental protocol also allowed the students to understand other aspects of NMR spectroscopy such as identification of impurities and its application in monitoring reaction progress. Results from a survey and a quiz conducted as part of the study clearly indicated that this hands-on approach not only helped to reinforce the theory learned in a lecture course, but also allow the students to realize the full potential of this important spectroscopic technique.

Keywords: General and Organic Chemistry, NMR Spectroscopy, Hands-On Experiment, Problem-Based Learning, Benchtop NMR Spectrometer

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1. Introduction

The introduction of experiments involving hands-on application of NMR spectroscopy to augment the lecture component of the course is challenging. In terms of instrumentation, access to conventional superconducting NMR spectrometers are often limited and stratified since postgraduate research students and research staff are often prioritized over undergraduate students. Moreover, such experiments are time-consuming in view of the overall acquisition time at the laboratory grouping level (average of five minutes of machine time per student per sample). While exploring a viable alternative, we came upon a type of NMR spectrometers that have emerged recently- the benchtop. The advent of these spectrometers with their compact footprint and relatively shorter acquisition time (average one minute per sample per student) provided an avenue for achieving the aforementioned pedagogical objectives. Additionally, these low-field spectrometers (typically in operating frequency range of 42-100 MHz) are considerably more affordable and easier to maintain than their high-field counterparts.

We herein present the details of a newly-designed undergraduate NMR spectroscopy laboratory experiment utilizing a benchtop NMR spectrometer to provide students with a strong foundation in both the practical and theoretical

aspects of NMR spectroscopy while inculcating valuable soft skills such as teamwork and collaborative learning.

2. Pedagogical Philosophy

The underlying pedagogical philosophy behind our experimental design was for the students to engage both in deep and active learning. It has been shown that deep learning has many benefits over surface learning [4,6]. One of the key facets of the deep learning educational paradigm is to relate theoretical knowledge [3] that is already known to a real-world situation. In addition, active learning, as proposed by Bonwell [3], has shown to have a positive long term influence on students [9]. As such, we felt that the merger of these aspects of learning would be extremely beneficial to the students in the context of NMR spectroscopy. We proposed that if we could simulate a realistic problem and have students successfully apply the knowledge they already possess from lectures in solving it, the students would gain a deeper understanding while acquiring the associated hands-on skills.

Overall, the experiment aims to train the students to: (1) practice proper NMR sample preparation; (2) acquire the problem solving skills necessary to enforce the theoretical aspects of NMR taught in lectures; and (3) facilitate meaningful learning through practice.

3. Summary of Procedure

An overview of the laboratory workflow for the newly-designed experiment is shown in Figure 1. Subsequent to attending approximately six hours of lectures that cover the theoretical aspects of ^1H NMR, students are first expected to complete a pre-laboratory assignment prior to their laboratory session. It must be noted that these students have completed their stipulated freshmen lectures in introductory organic chemistry and are therefore already familiar with the common functional groups as well as the concept of isomerism.

During the laboratory session, students will proceed to prepare the NMR sample, acquire data using the benchtop NMR spectrometer and process it under the guidance of a teaching assistant. Upon conclusion of the laboratory session, the students are required to complete a prescribed post-laboratory exercise. The experimental worksheet, containing the pre-laboratory assignment and post-laboratory exercises, as well as the laboratory manual are included in the supporting information.

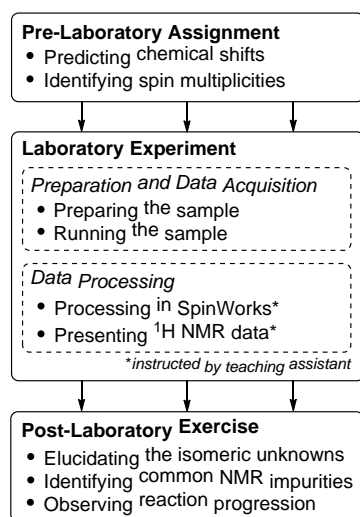


Figure 1. Workflow for Experiment

3.1. Pre-Laboratory Assignment

Prior to the laboratory experiment, students are expected to complete a short exercise pertaining to the fundamentals of ^1H NMR spectroscopy. The questions are meant to highlight the concepts that students would need in order to solve problems in the post-laboratory exercise. The questions focus primarily on prediction of chemical

shifts and spin multiplicities of protons within the molecule.

3.2. Laboratory Experiment

The actual experiment consists of two segments: (1) sample preparation; and (2) data collection on the benchtop NMR spectrometer. Data collection is followed immediately by the data processing element in the computer laboratory.

Each laboratory session consisted of approximately 30 to 40 students to be split into five sections (with the other four sections conducting separate experiments) of about 6 to 8 students. The size of each laboratory section was kept small due to the limitation in the number of benchtop NMR spectrometer (in our case, one) and processing workstations (16 standalone computers within our teaching laboratory). Students within the section are then further divided into pairs, with each pair being provided a set of two isomeric unknowns and their simplified molecular formula. It should be noted that although the experiment can be conducted at the individual level, the rationale in pairing students is to allow them to partake in collaborative learning. Such an approach promotes meaningful discussions and teamwork which has been shown to be far superior when compared to one where students learn as individuals [7,8,11]. In practice, the section size could be scaled up to accommodate a larger number of students, subjected to the availability of spectrometers and workstations.

A group size of 10 students usually takes 40 minutes for sample preparation (5-10 minutes per pair), data acquisition (2 minutes per pair) and cleaning up (5-10 minutes per pair). An additional hour may be required for data processing and assisted analysis of the obtained data. For larger class sizes, it is suggested that at least two benchtop NMR spectrometers and 30 workstations to be available for the experiment.

In terms of the choice of unknowns and sample preparation, it needs to be highlighted that the benchtop NMR spectrometers are slightly disadvantaged by its lower sensitivity and resolution. To overcome such issues, solutions of the unknowns were made in higher concentrations (0.1-0.3 M) than typical ^1H NMR samples (0.01-0.05 M). In addition, we were concerned about the chemical shift distribution and second-order effects that may make it difficult for the students to analyze the ^1H NMR spectra. As such, complex motifs (i.e. long alkyl chains, cyclic compounds and/or *ortho*- or *meta*-substituted rings) or uncommon functional groups, are avoided in the experiment.

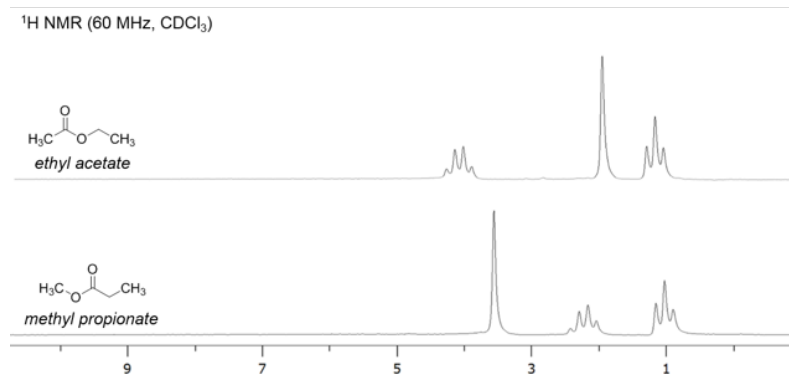


Figure 2. Processed ^1H NMR spectra of the $\text{C}_4\text{H}_8\text{O}_2$ isomers

3.3. Post-Laboratory Exercise

Students complete the experiment with a post-laboratory exercise aimed to equip them with the skills needed to: (1) elucidate structures; (2) recognize NMR trace impurities such as solvents; and (3) develop reasoning for other applications of NMR. The post-laboratory exercise is thus meant to further train the students to gain an appreciation of the critical roles that factors such as impurities/solvent generated resonance signals can play in an actual synthesis scenario.

4. Results & Discussion

The newly-designed laboratory experiment has been conducted for more than 1000 students over five semesters, supervised by eight different teaching assistants, as part of the undergraduate general chemistry curriculum at Nanyang Technological University, Singapore. In the case of the largest cohort of 280 undergraduate freshmen chemistry students, the students were divided over 40 three-hour laboratory sessions over the course of the semester.

The current experiment format was a significant modification of the previous that relied solely on the interpretation of spectra that was provided to the students. However, in order to assess if students benefited from this experiment, we conducted a quiz for 51 students, half of whom have successfully completed the laboratory session and compared their scores against a control group consisting of the remaining students who have yet to partake in the experiment. Both groups had gone through the same lecture session pertaining to NMR where they were taught the theoretical aspects. This quiz had four components: (1) NMR fundamentals (involving chemical shifts and spin multiplicity); (2) distinguishing isomers from ^1H NMR spectra; (3) determination of trace NMR impurities; and (4) monitoring reaction progress. The overview of the results of the quiz is shown in Figure 3.

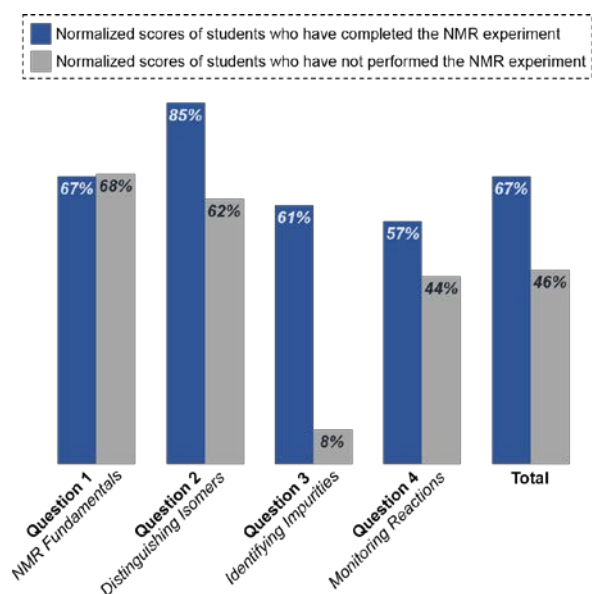


Figure 3. Comparison of performance of students who have performed unknown identification experiment vs those who have not

The question relating to NMR fundamentals was included to act as a control to determine the level of

understanding of NMR theoretical concepts within the students purely based on the lecture. The results clearly indicate that although students had a similar theoretical competency (Question 1), those who had hands-on experience *via* the laboratory experiment were more adept at applying their knowledge to a real world situation.

The striking difference in scores for Question 3 showed that students with this experience were able to link their prior knowledge to an actual spectrum, an aspect that may be difficult to emphasize during a lecture course (especially if class size is large). The ability to apply theory in a practical dimension shows at least a relational level of understanding according to the SOLO taxonomy proposed by Biggs [1].

5. Conclusion

This laboratory experiment thus provides students with a valuable hands-on experience of the various components that constitute a typical NMR-based molecular characterization protocol and its application in the identification of unknown molecular samples. Moreover, students learnt that spectra obtained may contain contaminants and learnt how to disregard such signals during structural analysis through problem-based learning. The benefits of this experiment, coupled with the accessible and extreme user-friendly nature of the benchtop NMR spectrometers makes this a very efficient way of teaching the many facets of NMR spectroscopy, both from the theoretical and practical perspective. It is envisaged that with the increasing availability and affordability of benchtop NMR spectrometers, and the options for 2D NMR and the observation other nuclei, new experiments can be designed to complement experiments that are already in the literature [2,5,11,12].

Acknowledgements

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Notes

The laboratory manual for the experiment, along with its relevant worksheet are available in the supplementary information. The list of isomeric unknowns selected by the authors can be obtained at request from S. A. Pullarkat or S. D. Riegel.

SpinWorks is a NMR processing software that provides basic off-line processing of 1D and 2D data. It is a freeware and can be downloaded from <http://home.cc.umanitoba.ca/~wolowiec/spinworks/> (accessed 23 May 2016). Data exported is compatible with SpinWorks 4.0 or higher.

References

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Supplementary Information (SI)

EXPERIMENT 1

¹H NUCLEAR MAGNETIC RESONANCE

(experiment to be done in pairs)

Introduction

Nuclear magnetic resonance, or NMR, has become an invaluable tool to many chemists, biologists and even clinicians. To date, the development of NMR has led to the awarding of three Nobel Prizes, namely to Rabi in 1944, Bloch and Purcell in 1952, and Ernst in 1991.

Knowledge of the physical basis behind NMR is essential in order to facilitate its use in analysis. All nucleons (as well as all elementary particles and composite particles for that matter) possess an intrinsic form of angular momentum known as spin. If the spin of an atomic nuclei is non-zero, it is able to absorb radio frequency energy in the presence of a magnetic field. In addition, the radio frequency (RF) pulse must be at a frequency characteristic to the identity of the nuclei. The spin quantum number of a nuclei can either be zero, and integer or half-integer. For pedagogical purposes, the rest of the description will center around nuclei with a spin quantum number (I) of $\frac{1}{2}$, covering a majority of spectroscopically useful nuclei such as ¹H, ¹³C, ¹⁹F and ³¹P.

For nuclei with a spin quantum number of $\frac{1}{2}$, there are two possible spin states, $m = \frac{1}{2}$ and $m = -\frac{1}{2}$. In the absence of a magnetic field, these states have the same energy and are said to be degenerate. However, in the presence of a non-zero magnetic field, the two spin states no longer are degenerate and are split by an energy, ΔE , proportional to the strength of the magnetic field. When nuclei are subjected to an RF pulse, the energy is absorbed by nuclei, allowing spins to flip from the lower energy state to the higher energy state. Simultaneously, energy is emitted by spins flipping back to the lower energy state. The population difference between the upper and lower state results in the signal in NMR spectroscopy. The sensitivity of NMR is due to the fact that very small population differences between the two states can be detected.

Of the above-mentioned nuclei, ¹H is the most sensitive nuclei because of high natural abundance and its large Larmor frequency. Despite a small chemical shift range, its signals are sharp and are characteristically useful by virtue of their chemical shift. In addition, the multiplicity of the splitting allows for deduction of the chemical environment of each specific nuclei of interest.

In a routine NMR spectrum, the ratio of the number of protons of a pure sample can be obtained directly from the integrals of each signal, provided that there is good resolution and no overlap.

However, it must be noted that there are a plethora of uses for NMR. In chemistry laboratories, NMR has been used for the determination of compound purity, tracking of reaction progress, in addition to structural elucidation of unknowns, which will be undertaken in this laboratory experiment.

In this experiment, you will learn how to prepare a sample for NMR analysis, learn to acquire the spectrum on a benchtop NMR spectrometer and, finally, utilize SpinWorks for processing of the acquired spectrum. Through the experiment and the corresponding worksheet, you will understand that, in reality, spectra generated are not always going to display clear, first order splitting and that impurities may interfere with compound identification.

The Experiment

You will work in pre-allocated pairs. Both of you will receive two compounds, which are isomers of each other. Under the supervision of a teaching assistant, you will begin by dissolving the sample in an appropriate deuterated solvent

(CDCl₃ in this case). It is essential that the solution is homogenous and must be filled to an appropriate height. After which, you will proceed to acquire the spectrum with the preset parameters. The raw, unprocessed data will be exported to flash drives and brought to the computer terminals for data processing with the help of the teaching assistant.

EXPERIMENT 1 ¹H NUCLEAR MAGNETIC RESONANCE

EXPERIMENTAL WORKSHEET

Name	Report
Date	
Date of Submission	/80
Marker's Comments	

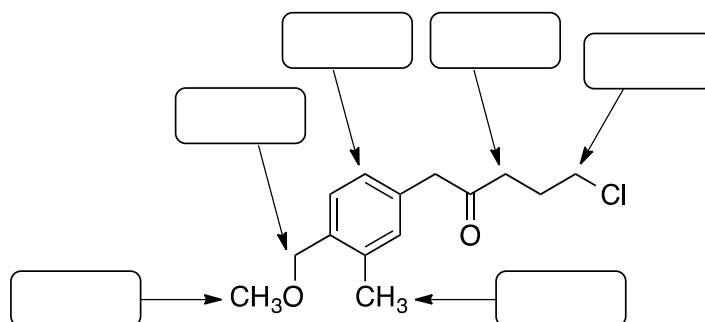
Risk Assessment

Identify one hazard associated with this experiment and give one reasonable precautionary measure.

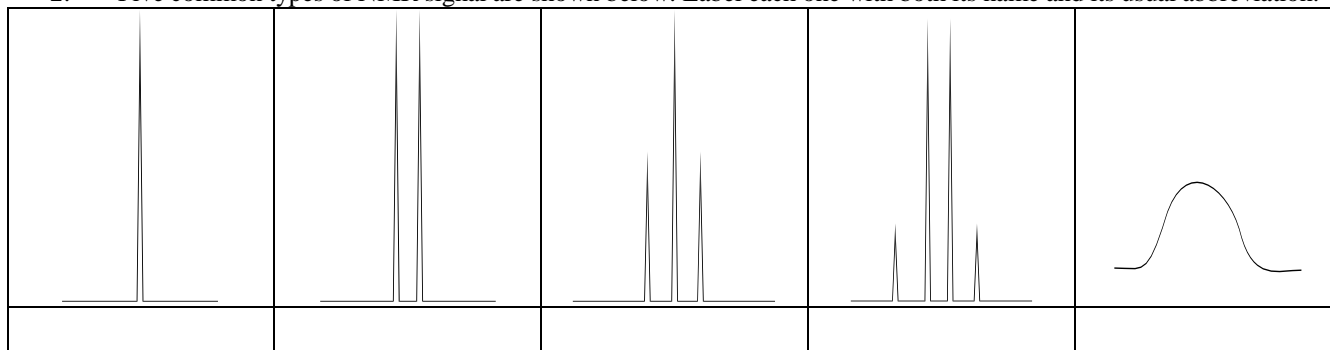
	Hazard	Precaution
1		

Pre-Laboratory Assignment

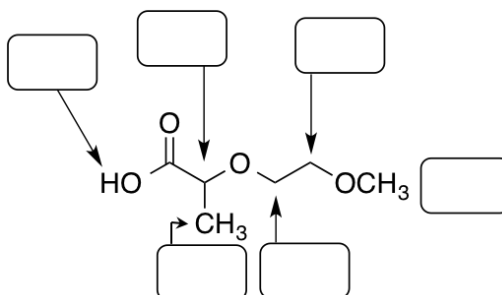
1. Predict the chemical shifts of the indicated protons in the molecule below by writing the values in the boxes.



2. Five common types of NMR signal are shown below. Label each one with both its name and its usual abbreviation.



3. The molecule below contains each of these types of signal (ignore any effects due to stereochemistry). In each box that points to a proton, write the abbreviation of its NMR signal.



Which type of signal appears twice?

Discussion

You are provided with two unknown samples which are isomers of each other. Prepare and run ^1H NMR samples of your unknowns. Thereafter, process your data using SpinWorks.

Attach your processed ^1H NMR spectra with your worksheet.

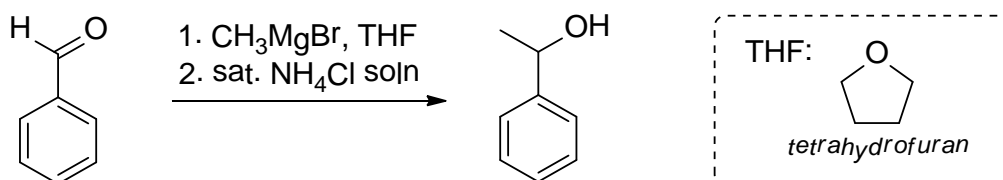
Determine the identity of the unknowns allocated to you by drawing its structure below. You are expected to justify your answer.

Unknown Samples &

Molecular Structure	Explanation
A	
B	

Post-Laboratory Exercises

- A student conducts an experiment using the synthetic protocol shown below.



A solution of benzaldehyde (1.0 mmol) in anhydrous tetrahydrofuran (5 mL) was added dropwise to a stirring solution of methylmagnesium bromide solution (1.1 mmol) in the same solvent (3 mL) at 0 °C under inert atmosphere. The reaction mixture was then left to stir at room temperature for 6 h. Saturated ammonium chloride solution (5 mL) was added slowly to the resulting mixture before extraction with ethyl acetate (3 x 10 mL). The organic layer was then washed with brine (20 mL), dried with MgSO_4 , filtered and evaporated to dryness.

Upon completion of the experiment, he obtained the following ^1H NMR spectrum (Figure 1a) for his product. He then compared his spectrum against a ^1H NMR spectrum of the pure product (Figure 1b) and noticed that there are impurities.

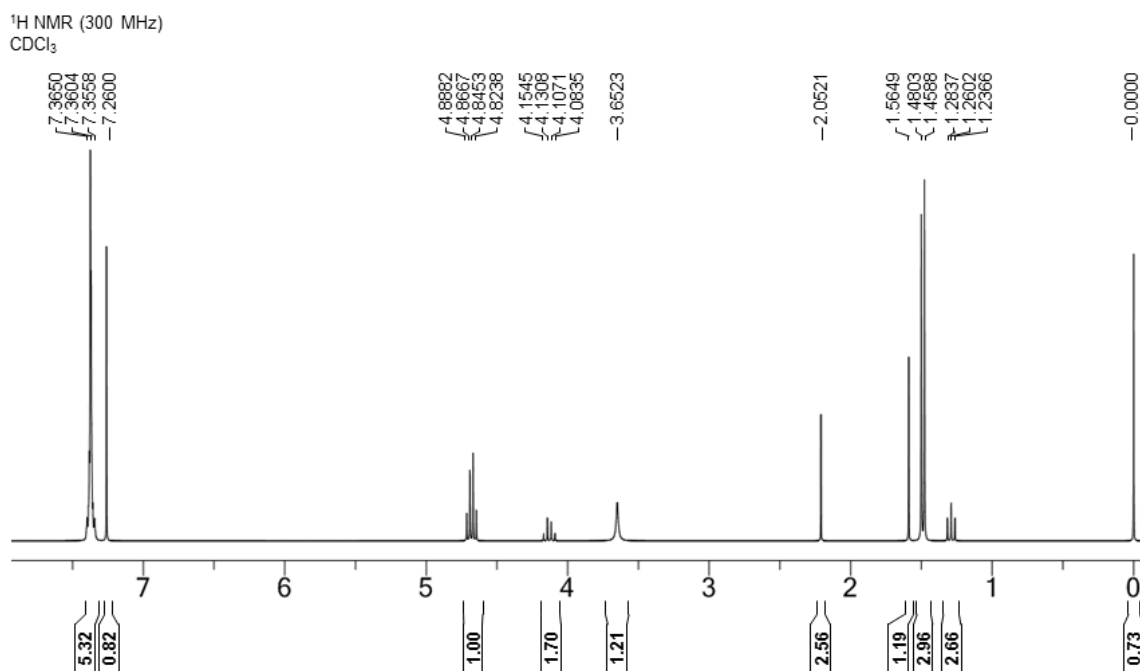


Figure 1a. Spectrum of product obtained by student

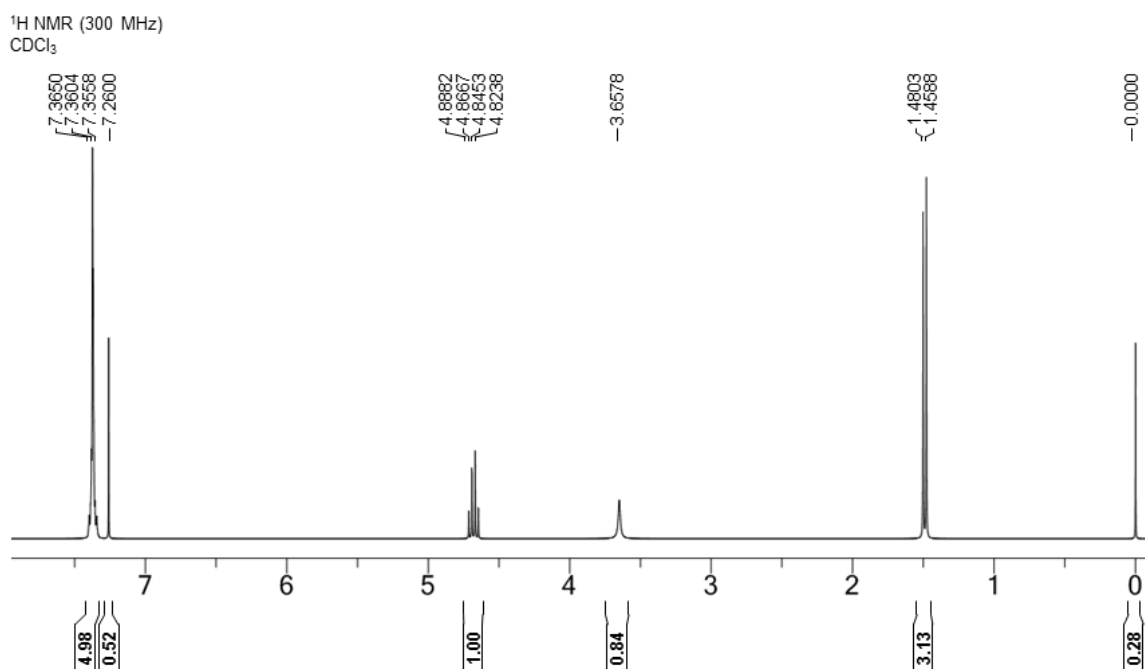
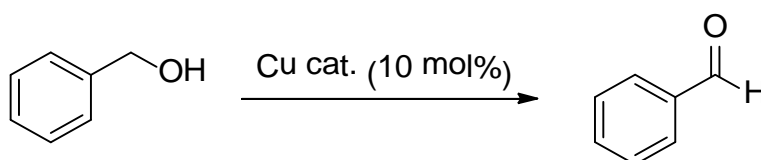


Figure 1b. Pure product spectrum

- State the identities of the impurities with its characteristic peaks and suggest its source.
 - Using the spectrum in Figure 1b, describe the signals in journal format.
- Other than structural elucidation, NMR spectroscopy can also be used to monitor a reaction. A student performed a reaction as shown below.



Suggest a method to determine the completion of the above reaction using ^1H NMR spectroscopy.

Experiment Feedback Nuclear Magnetic Resonance Quiz

Instructions to Students

This quiz serves to be a feedback for the nuclear magnetic resonance experiment. **Results of this quiz will not influence your grades** for tests, examinations or laboratory reports. In addition, this survey, or parts of it, may be utilized in a scientific publication.

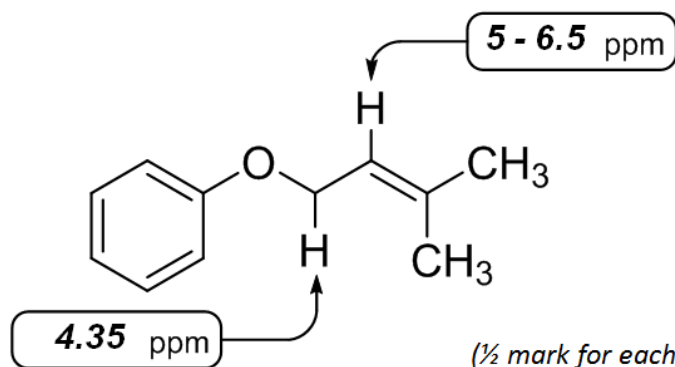
The survey contains **FOUR (4)** questions and comprises of **FIVE (5)** pages. Please answer all questions. A copy of the NMR Experiment Supplementary Booklet (NESB) is also provided.

Matriculation Number:

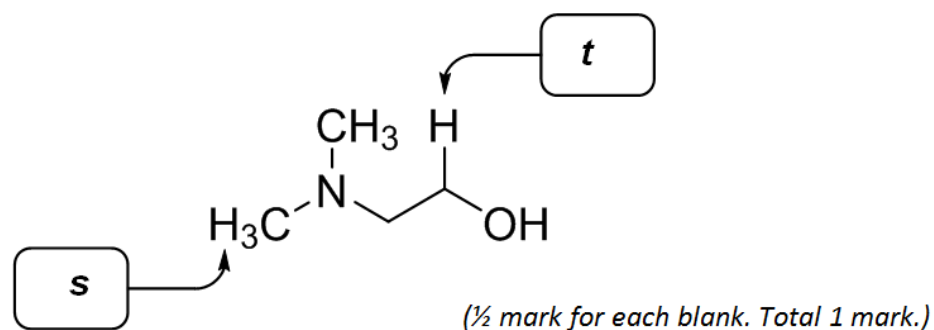
S	O	L	U	T	I	O	N	S
---	---	---	---	---	---	---	---	---

Matriculation number is for administrative purposes only. The quiz will not influence your grades.

- Chemical Shifts and Spin Multiplicities**
 - Predict the **chemical shifts** of the indicated protons of the molecule below by writing the values in the boxes.



- (b) Write the **abbreviation** of the type of NMR signal (i.e. single (s), broad singlet (br s), doublet (d), triplet (t), quartet (q), doublet of doublet (dd), etc.) observed for the molecule in the boxes below.

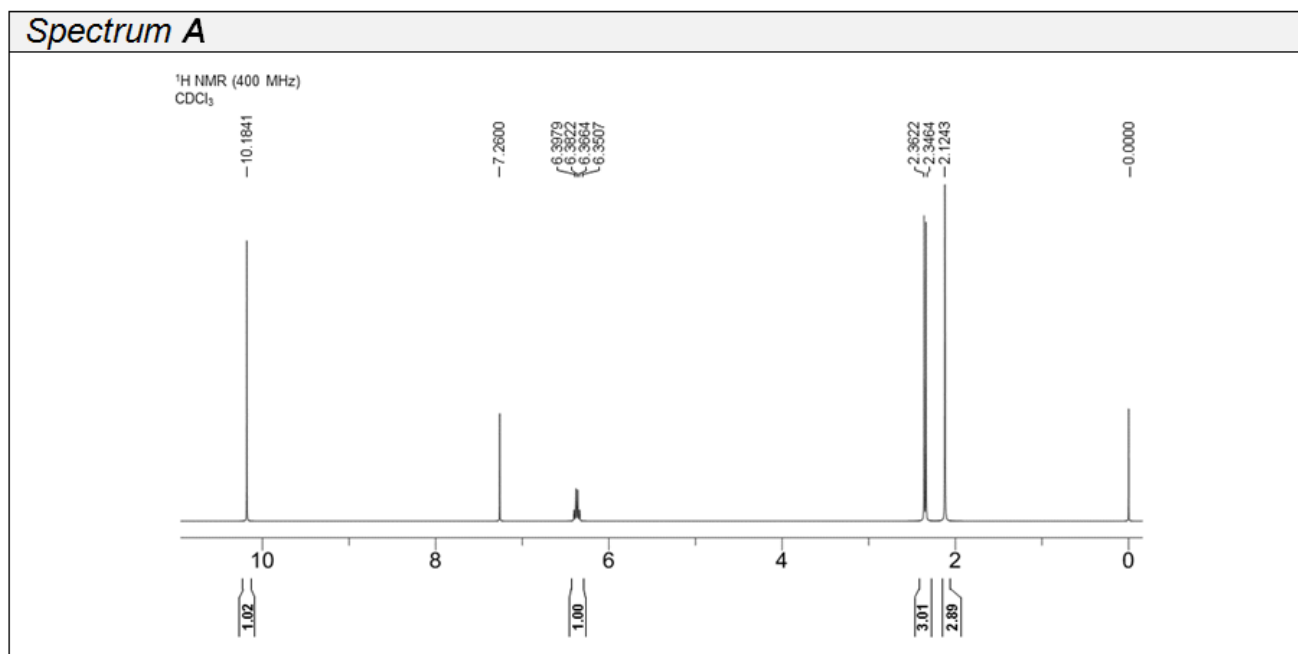


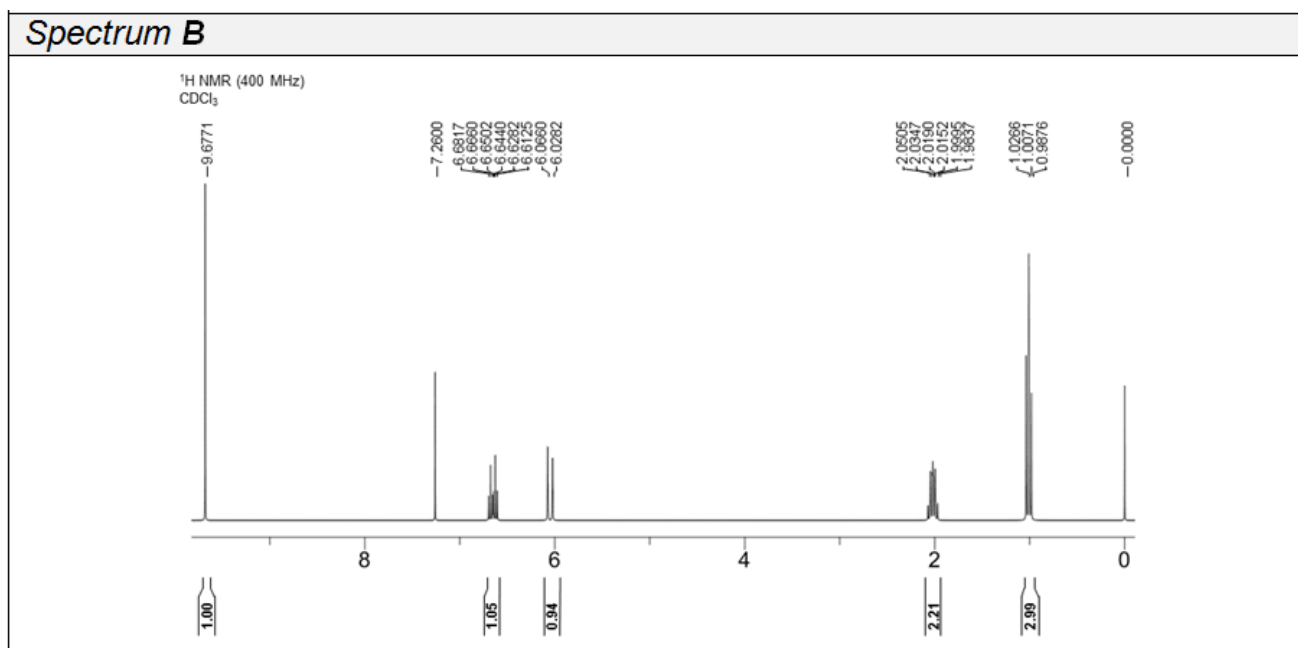
2 Distinguishing Isomers

This question requires you to distinguish between isomers. Match the correct isomer to the correct spectrum.

- (a) (*E*)-2-Methylbut-2-enal and (*E*)-Pent-2-enal (Total 1 mark.)

<p>(<i>E</i>)-2-methylbut-2-enal</p>	<p>(<i>E</i>)-pent-2-enal</p>
spectrum A	spectrum B

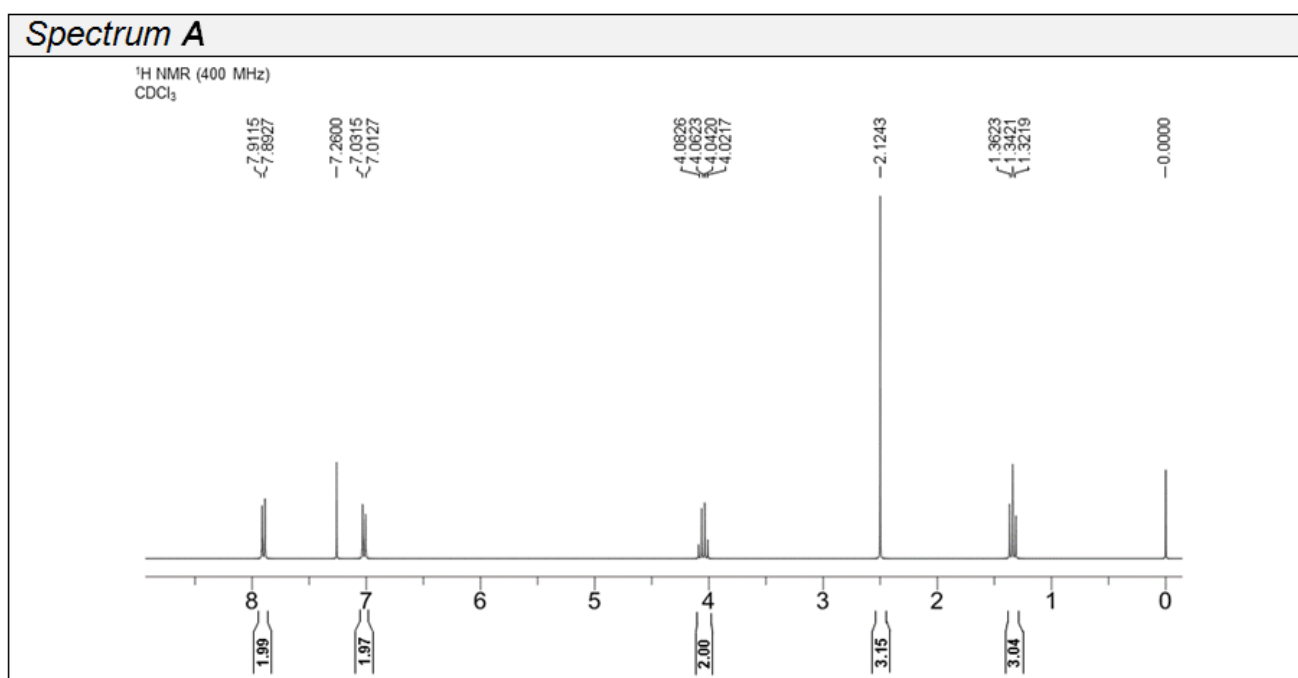


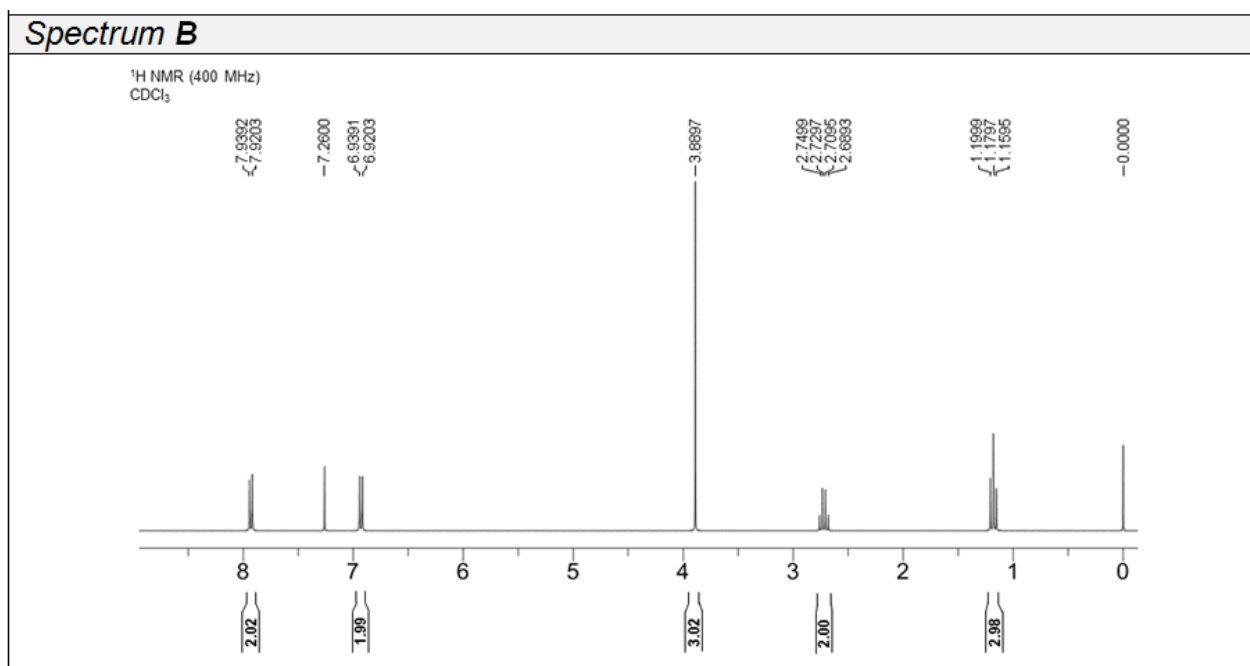


(b) Methyl 4-methylbenzoate and 1-(4-Ethoxyphenyl)ethan-1-one

(Total 1 mark.)

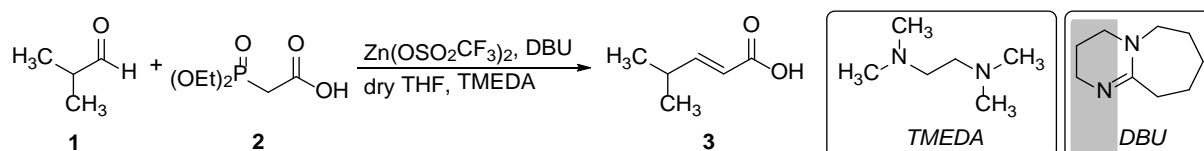
 <chem>CCc1ccc(cc1)C(=O)OC</chem> <i>methyl 4-methylbenzoate</i>	 <chem>CCOCc1ccc(cc1)C(=O)C</chem> <i>1-(4-ethoxyphenyl)ethan-1-one</i>
spectrum B	spectrum A





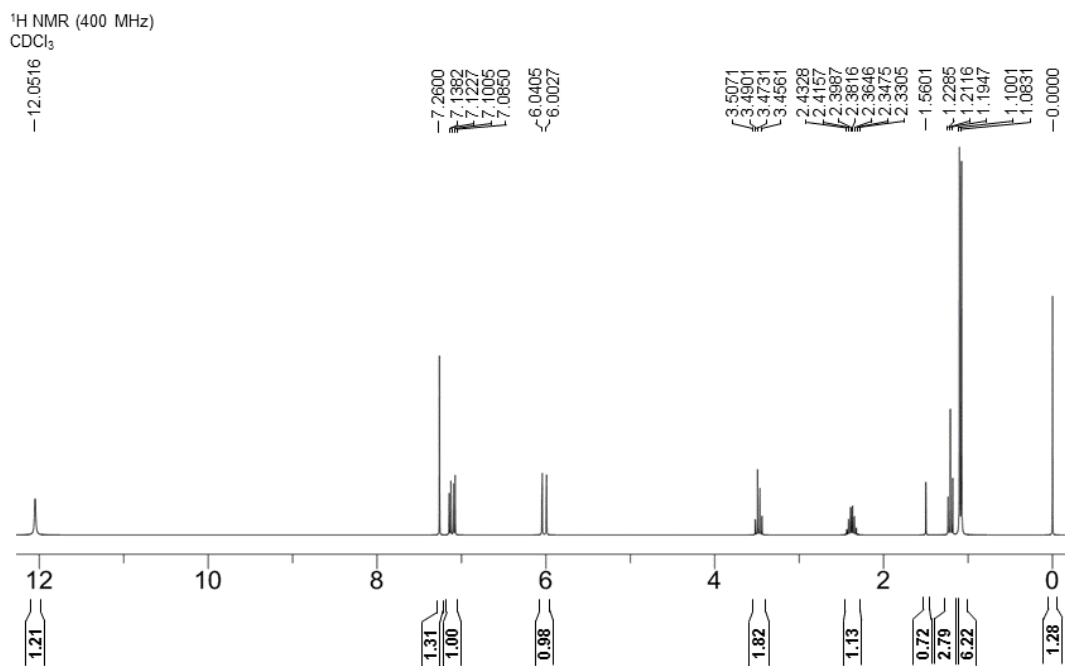
3 Identification of Common ¹H NMR Trace Impurities

A student conducts an experiment using the synthetic protocol shown below.



A solution of $\text{Zn}(\text{OSO}_2\text{CF}_3)_2$ (2.0 mmol) and TMEDA (1.0 mmol) in anhydrous THF (5 mL) was added dropwise into a stirring mixture of phosphonate **2** (1.0 mmol) in the same solvent (5 mL) under inert conditions. DBU (4.0 mmol) was then added to the solution, followed by aldehyde **1** (1.0 mmol). The reaction mixture was left to stir at room temperature for 12 h. An aqueous solution of HCl (1 N, 5 mL) was added to destroy remaining DBU and TMEDA. The resulting mixture was extracted with diethyl ether (3 x 10 mL), dried with MgSO_4 , filtered and evaporated to dryness.

Upon completion of the experiment, he obtained the following ¹H NMR spectrum and noted that there exist some impurities in his spectrum.



State the **identities** of the impurities and suggest its **source**.

(Do not state residual solvent peak of CDCl_3 and TMS as impurities! You may use the supplementary information provided to aid you in the determination of the impurities.)

Water (From hydroscopic CDCl_3 or From organic layer that is not sufficiently dried)

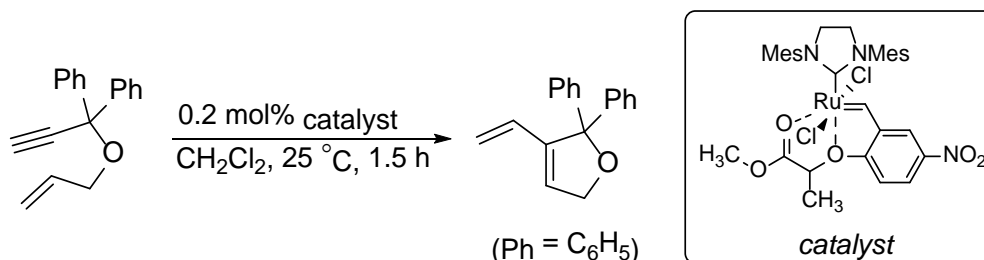
Diethyl Ether (From extraction procedure)

($\frac{1}{2}$ mark for each impurity, $\frac{1}{2}$ mark for valid reason for each impurity. Total 2 marks.)

4 Monitoring of Reactions

Other than structural elucidation, NMR spectroscopy can also be used to monitor a reaction.

A student performed a reaction as shown below.



Suggest a method to determine the completion of the above reaction using ^1H NMR spectroscopy.

Track the disappearance of the terminal alkyne CH singlet between 1.75 to 3.25 ppm.

(1 mark for disappearance of terminal alkyne proton, $\frac{1}{2}$ mark for spin multiplicity (singlet), $\frac{1}{2}$ mark for either exact or expected range of alkyne proton chemical shift. Total 2 marks.)

Nuclear Magnetic Resonance Experiment Quiz Results

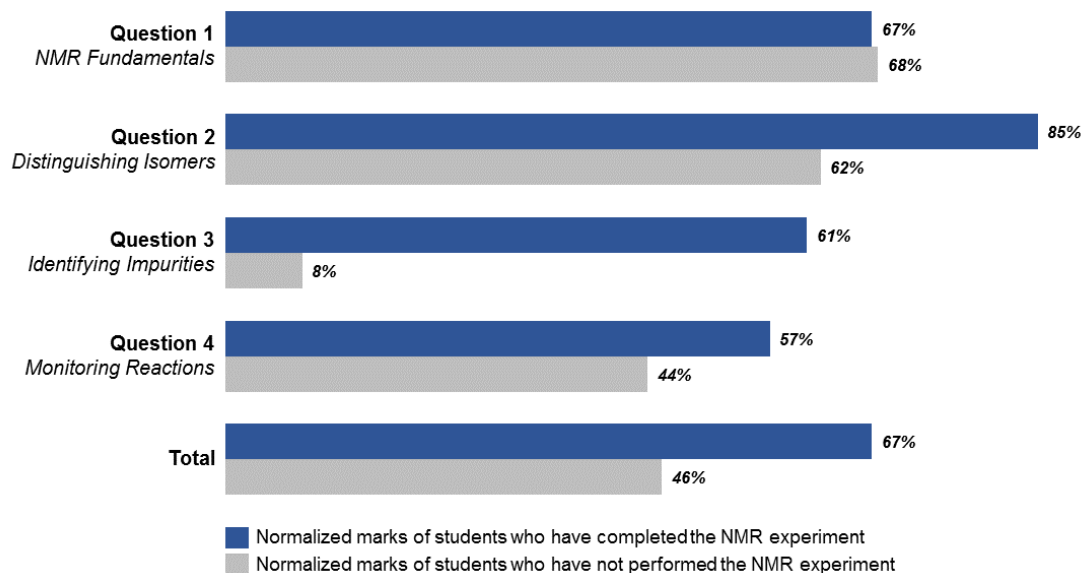
Scores

Average Scores

		Qn 1 (2)	Qn 2 (2)	Qn 3 (2)	Qn 4 (2)	Total (8)
Students who have completed the NMR experiment	<i>average</i>	1.35	1.69	1.21	1.13	5.38
	<i>% average</i>	67%	85%	61%	57%	67%
Students who have not performed the NMR experiment	<i>average</i>	1.36	1.24	0.16	0.88	3.64
	<i>% average</i>	68%	62%	8%	44%	46%

^aTotal score of question or component in parenthesis. ^b% average is calculated from the average score over the total score of the question or component.

^cAverage scores are rounded off to two decimal places, and % averages are rounded off to the nearest whole number.



Individualized Scores

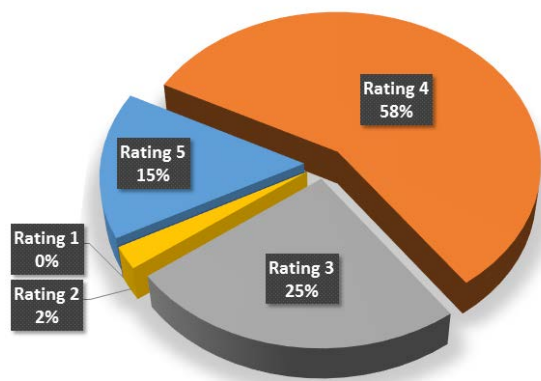
Completed NMR Experiment				
<i>marks scored by students</i>				
Marks	Q1	Q2	Q3	Q4
0	1	2	5	9
0.5	2	0	2	1
1	8	4	6	2
1.5	8	0	3	2
2	7	20	10	12
Total	26	26	26	26

Have Not Performed NMR Experiment				
<i>marks scored by students</i>				
Marks	Q1	Q2	Q3	Q4
0	0	6	20	7
0.5	4	0	4	1
1	5	7	0	11
1.5	10	0	0	3
2	6	12	1	3
Total	25	25	25	25

Results for Survey on Experiment

Total Participants: 169

- On a scale of 1 to 5, with 5 being the most informative and 1 being the least informative, please rate how much you learnt from this experiment?



5	4	3	2	1
26 (15.4%)	97 (57.4%)	42 (24.8%)	4 (2.4%)	0 (0%)

Average rating: 3.86

- Has the experiment helped in improving your understanding of ^1H NMR?

Yes	No
151 (89.3%)	18 (10.6%)

- Are you able to relate theoretical knowledge of ^1H NMR gained from the lectures to the experiment?

Yes	No
147 (87.0%)	22 (13.0%)

- Did the experiment highlight the alternate uses of NMR, i.e. reaction monitoring, assessment of purity of products?

Yes	No
150 (88.8%)	19 (11.2%)

- Did the experiment illustrate how NMR may be used to differentiate isomers?

Yes	No
137 (81.1%)	32 (18.9%)

- Did you face any difficulty in using the benchtop NMR machine interface to acquire data?

Yes	No
2 (1.2%)	167 (98.8%)

- Did you find NMR sample preparation straightforward?

Yes	No
162 (95.9%)	7 (4.1%)

- Would you feel confident preparing a sample, acquiring NMR data and working up a spectrum in the future?

Yes	No
151 (89.3%)	18 (10.7%)

Other Comments.

- Helped a lot in NMR theory understanding
- Awesome experiment! Love it!
- The experiment is useful.