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Citrus Quality Control: An NMR/MRI Problem-Based Experiment

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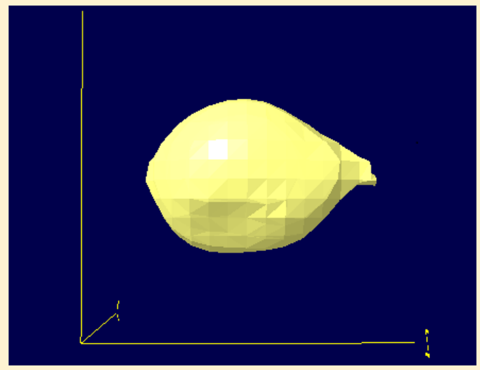
Supporting Information

ABSTRACT: An experiment seated in an industrial context can provide an engaging framework and unique learning opportunity for an upper-division physical chemistry laboratory. An experiment that teaches NMR/MRI through a problem-based quality control of citrus products was developed. In this experiment, using a problem-based learning (PBL) approach, students construct knowledge about the physical properties that make NMR and MRI possible, apply them to make decisions about experimental conditions, and write a final report in the form of a technical report for a fictional company. Implementation in a laboratory course demonstrated that the majority of students met the desired learning outcomes.



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Nuclear magnetic resonance (NMR) spectroscopy is an invaluable technique in determining connectivity, spatial, and dynamic information to characterize and isolate materials in molecular and biological studies.[1−3] Despite its importance, instruction on NMR spectroscopy often focuses solely on the interpretation of spectra, rather than the physical, quantum mechanical, and instrumental concepts that underlie the technique.[4] Experiments usually use a high-field instrument, and although it allows for exposure to state-of-the-equipment, students often only need to click a button to obtain a spectrum, and are not asked to consider the mechanics of the instrument.[5−7] Moreover, magnetic resonance imaging (MRI), as an extension to NMR, is often not included in the laboratory, even though it is a critical technique in the chemical, biological, and health sciences.[8,9] To resolve these issues, we describe the use of an Earth's magnetic field NMR/MRI benchtop spectrometer, the Terranova NMR/MRI,[9] which allows for students to explore concepts as well as interpret spectra.

The techniques of NMR and MRI have wide applicability; however, many of the current undergraduate experiments have students collecting spectra of compounds and collecting data with little to no context and are given each step to perform in data collection and analysis.[2,6] Therefore, this experiment was designed with a problem-based learning (PBL) structure in order to engage students and to encourage problem-solving and reasoning skills.[10,11] In PBL, students are given a compelling, authentic problem to guide their investigation, and they must ensure that they collect data that will allow for a solution. Although this technique of student-centered teaching is often done as a long-term, overarching project where students independently develop the experiment, the components can be used to develop a guided approach that allows for the learning experience to fit time constraints, in this case a two-week lab period.[12] In this experiment, to meet the student learning outcomes of explaining the physical, quantum, and instrumental concepts, students are not given the material up-front in an introduction in order to create a "need to know." As students work, they encounter guiding questions that connect the principles to prior concepts, or are given resources to explore that allow for construction of ideas about NMR and MRI. This allows students to synthesize the content that would be normally presented in lecture before they perform the experiment. Additionally, one of the key components of PBL is for the assessment to be authentic to the scenario.[11,12] As a result, students are asked to write a technical report to a company to address a quality control problem of citrus products, which also allows for practice in writing to a nontechnical audience.

Overall, this novel classroom-ready experiment provides an experience to build knowledge about the techniques of NMR and MRI in the context of a quality control issue from a fictional company. It was developed using accepted reformed teaching practices, tested by a group of graduate and undergraduate students, and implemented and evaluated with the target population in an upper-level laboratory course. Contained in the Supporting Information is the following: student guide with a problem statement, assignment descriptions, protocols, and guiding questions; instructor's guide with answers to questions, questions that may occur during experiment, and intervention points; a rubric with grading

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categories; and a sample student report from a student in the course. Point assignment is left to the individual instructor.

■ LEARNING OUTCOMES

The experiment aims to help students construct content and procedural knowledge about NMR and MRI spectroscopy. In this experiment, students will do the following:

1. Construct mathematical and physical models of the physical manifestation of the technique of NMR.
2. Compare and contrast the technique to other types of spectroscopy.
3. Acquire NMR and MRI spectra of water.
4. Explain and acquire the physical parameters of NMR (e.g., pulse durations, FIDs, Fourier transformations).
5. Apply the technique to a real-world problem.
6. Defend their protocol choices.
7. Write a final report in the form a technical report.

Additionally, learning objectives of scientific writing, team work, and critical thinking are embedded in the overall lab experience. The learning outcomes were obtained by the majority of the students when it was implemented.

The lab is designed to be implemented in an upper-level physical chemistry or instrumentation course. As such, it was assumed that students had some prerequisite knowledge on the technique of NMR and basics of spectroscopy. To be able to implement the current iteration of the lab, authors suggest that students have mastered these learning outcomes:

1. Explain NMR is a technique that studies nuclei in magnetic fields, and that hydrogen is often the nuclei of study.
2. Describe different types of spectroscopy and the approximate energy of electromagnetic radiation used in each type.
3. Explain how spectroscopy studies the interaction of light with matter, and is useful due to quantized energy levels.
4. Explain how data in NMR is first collected as an FID and then converted to the frequency-domain spectrum.
5. Use computers and understand the basics of Windows software.

■ EXPERIMENTAL OVERVIEW

The following experiment is designed to be done in groups of two to three students; however, the deliverables (lab reports) are expected to be submitted individually. It is a two-week lab (each segment taking 2 to 3 h), with short pre-laboratory work to be completed. Therefore, students need to receive the packet of information from the fictional company, CitrusTech, at least 1 week before the experiment is planned. The first page is a short problem statement, describing a need for a quality control protocol for their citrus fruit. In the experiment, the students acquire NMR and MRI data to successfully identify if the Terranova NMR/MRI is a valid and feasible way to perform nondestructive quality control. Many students will identify that the cost of this technique would likely inhibit companies from using the technique; however, the problem is representative of situations where nondestructive techniques could be warranted. Moreover, the activity requires students to provide financial and quality control evidence to support their reasoning. The experiment is segmented into parts to ensure that students build a connection between the physical manifestation of NMR, its signal, and the use of gradients to obtain MRI images.

Table 1. Timeline of Project and Expected Deliverables

Project Description	Deliverable	Due
Background research	Pre-Lab Week 1	3 days before week 1 experiment
Procedure development	Pre-Lab Week 2	3 days before week 2 experiment
Parameter and method development for NMR	Report 1	Start of experiment week 2
Response to problem statement	Technical Report	One week after week 2 experiment

The deliverables are outlined in Table 1, and this packet, titled "Student Laboratory Guide", is included in the Supporting Information. The pre-laboratory work is requested before the first week of laboratory work, as it allows the instructor and/or TA to evaluate if students have the conceptual understanding needed to be successful. This design allows for instructor intervention, if needed, before students use incorrect conceptions of theory or design an unsuccessful experiment.

Following the theory of PBL, the scenario, although more controlled and structured than would be in a company setting, follows expectations, language, and problem type that could be encountered in a company setting.[11] Each segment of the experiment is explicated in detail below.

Pre-Lab Week 1: Background Research

A key design component of this experiment is the construction of questions that encourage meaningful learning by making connections to prior knowledge and experience, and giving a context to learn the new material.[13] In the first pre-lab, students are provided a series of scaffolded questions that guide them in building a relationship between the magnetic field and energy differences of spin states (see Box 1). It also has students compare NMR to techniques with which the students have more familiarity (UV/vis and IR) while connecting spectroscopy to population and sensitivity.

Week 1: Parameter and Method Development for NMR

During the first week, students explore the experimental manifestations of the technique and learn the types of signal pulses to collect needed parameters (e.g., T_1, T_2, pulse duration). They also explore how parameters and signal change with the addition of a paramagnetic ion. A skeletal experimental guide, guiding questions, and instrument manual are provided to the student; however, the student groups must decide how they will collect the required information. The manual provides a rich literature basis for the experimental choices and theoretical ideas. It is referenced in the guides, but is not provided in the Supporting Information, as it is copyrighted. However, these manuals come with the instrument. Institutions adopting this lab in a different context would need to provide students different literature to explore as they encounter areas of confusion. Students have a short writeup with guiding questions that accompany this first part of their experimental work.

Pre-Lab Week 2: Procedure Development

Before the second week, students are asked to design a procedure that will use the MRI capabilities that will enable them to address their problem statement (see Box 2). Guiding questions are provided to assist students in their thinking about the technique; however, students are expected to use the manual and their knowledge of the instrument that was developed in the first week to decide how and what type of information to collect.

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Box 1. Example of Scaffolded Questions in Developing Student Understanding of the Phenomenon of NMR

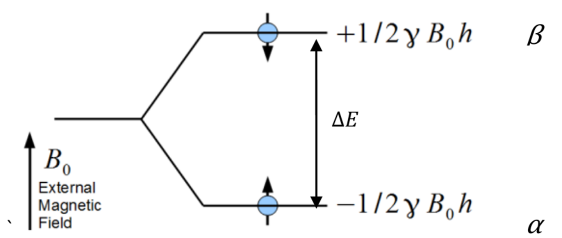

Figure 1: Energy level diagram for the spin ½ hydrogen(^{1}H) nucleus at a constant magnetic field. The external magnetic field determines the energy gap between the spin states, where γ is the gyromagnetic ratio, an intrinsic property of the hydrogen nuclear spin, and h is Planck's constant.

1. Figure 1 illustrates the effect of an external magnetic field on spin. Determine the equation for ΔE, the difference in energies of the two levels.
2. Using the NOAA Web site (http://www.ngdc.noaa.gov/geomag-web/#igrfwmm), determine the Earth's magnetic field based on your location.
3. Using the spectroscopic principle that resonance will occur when the energy of photons is equal to the energy separation, determine the frequency of the photons that will induce the transition between the nuclear spin energy levels in the Earth's magnetic field for a proton spin transition.
   Hint: Use questions 1, 2, $E = h\nu$ and $\gamma_H = 42.576 \times 10^6$ Hz/T
4. The Larmor frequency, the precession frequency, of nuclei placed in a magnetic field is equal to $\omega = \gamma B$. What is the Larmor frequency of hydrogen nuclei in the Earth's magnetic field?
5. What is the relationship between answers to questions 3 and 4? Why is this the case?

Week 2: Response to Problem Statement

In the second week of the experiment, two lemons, one that has been previously frozen and a regular lemon, are provided to the students as samples. The students use their planned procedure and user manual to acquire any needed images. Their final report is a technical report that is to be written in a style appropriate for scientists and financial analysts.

Equipment

Magritek's Terranova-MRI is used for this experiment. Information about the instrument and pricing can be found at their Web site.[9] A 500 mL water sample, three 500 mL solutions of Cu^{2+}, and citrus samples are also required.

■ HAZARDS

This experiment has no expected hazards; however, proper protocol for laboratory attire and behavior should always be followed.

■ RESULTS AND DISCUSSION

This experiment was performed by groups of two undergraduate students during a 400-level chemical measurements laboratory course. This course occurs after the students have completed a full-year of physical chemistry. The students are expected to transfer their knowledge, as well as use resources they obtained in their physical chemistry course and other upper-division courses, to address the questions posed in the experiments. In total, four students in the course performed the experiment. This was a limitation of the class size; only four students were enrolled in the course. However, prior to implementation, the experiment was piloted with other undergraduate and graduate students to ensure clarity of directions and questions. These data are not presented here, as these students were not the target population, and their data were used for refinement of the experiment. However, the piloting with other students and their success doing the lab lends further evidence to the quality of the experiment.

IRB approval and consent by the students was provided to allow for collection and retention of student work and for observation to occur while performing the experiment. The students were able to collect spectra and clearly explain the manifestations of the NMR signals and parameters. They were also able to use MRI imaging to justify the use of the technique as a nondestructive quality control technique for a fictional company.

Over the two-week period, students acquired multiple NMR/MRI spectra for different samples. Figure 1 illustrates a spectrum collected by one of the students that allowed him to address the problem statement. In his text, he was able to describe the change that occurs to the fluid-filled sacs in the carpels of the lemon. When ice crystals form, they puncture these, and the concentration of the water becomes more distributed, rather than segmented. The students had to determine what parameters they would need to collect to be able to

Box 2. Problem Statement

You are hired by a citrus company, CitrusTech, to develop a nondestructive quality control procedure for their lemons using their Terranova NMR/MRI instrument. CitrusTech operates by purchasing lemons from many different lemon farmers, combining the products, putting their name on them, and then selling the lemons around the country. Although the lemons come from different farms, each batch needs to be of the same quality. One issue, especially this past season, is that customers have been complaining about lemons that are quite mushy after purchase. Although the lemon juice seems to be fine, slices for beverages are impossible and restaurant vendors are threatening to discontinue their service with CitrusTech. After some investigation, it is discovered that many farms, especially in the Northern regions, had frosts very late in the season. CitrusTech thinks that this may be related to their quality control (QC) problem.

You will have 2 weeks to gather the data that you will need to help CitrusTech with their problem. CitrusTech is providing you a lemon from a farm that had a late frost and one that did not. *The project guidelines page gives instructions for the deliverables that are expected from CitrusTech.*

Although taking images with MRI is the ultimate goal of the experiment, you will first need to run NMR experiments to determine the parameters needed for the software and instrument to run the MRI. Moreover, part of your hiring contract is to be able to explain the procedure to the company and their technicians so that the process can be done internally after your contract. Therefore, it is expected that you be able to explain the purpose of each of your steps and the concepts of the technique. The company expects deliverables by the deadlines shown in Table 1.

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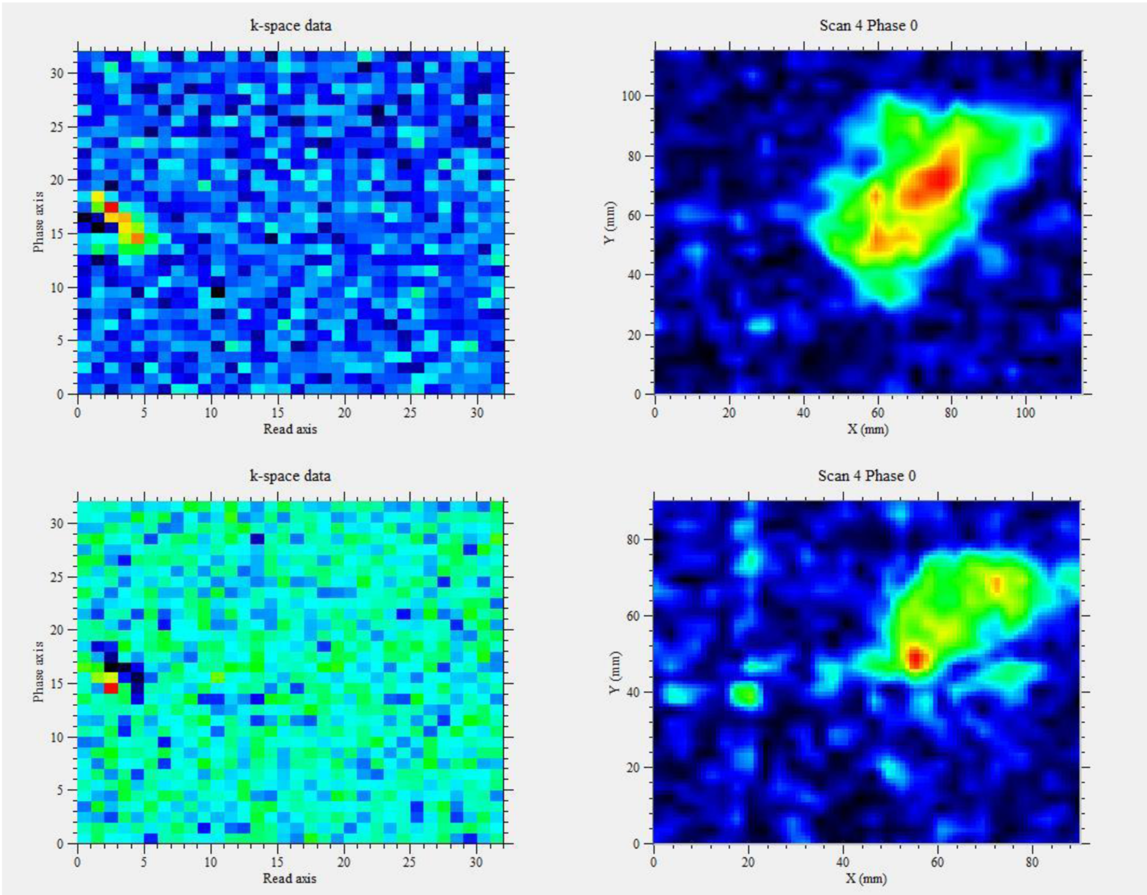

Figure 1. Example student spectra and their figure caption from the technical report, which shows the difference in MRI of a lemon exposed to frost and not exposed. Students are asked to follow ACS guidelines for figures and tables.

successfully acquire an MRI image and make decisions about the number of scans and orientation to be able to properly address the problem statement.

The rubric and teaching guide performed well, and provided adequate information to be able to assess the student learning gains. The rubric was able to distinguish the differences in student achievement and guide students in using ACS format and scientific language. The teaching guide provided enough supporting information to be able to guide students when questions and issues arose. In addition, data were collected during the course of the experiment on students' questions and challenges which helped to shape the instructor's guide to assist with future implementations of the lab. Moreover, students seemed to struggle the most with the development of the MRI protocol. This was not surprising, since it was the most open-ended part. As a result, additional leading questions were developed to assist students in this process.

■ STUDENT LEARNING OUTCOMES

The major learning objectives are illustrated in Table 2. The majority of students met all objectives at an *adequate* or *excellent* rating. Failure was marked as not attempting or giving a nonscientific answer. Adequate success occurred when students answered most of the questions correctly, but either failed to defend their answers or had minor scientific errors. An *excellent* was given when students completely demonstrated the learning objective and provided rationale. The Supporting Information provides a rubric for the experiment, as well as a teacher's guide with answers to the questions. No strict point guide is provided, as this is dependent on the instructor; however, during implementation, most of the points were awarded for the final technical report. The pre-laboratories, designed to be check points for success, were given the least

Table 2. Achievement of Learning Outcomes[a]

Learning Objective	Fails	Adequate	Excellent
Develop relationship between FID and frequency graph			4
Describe pulse duration purpose and differentiate between relaxation times.			4
Compare and contrast relaxation constants			4
Draw and describe basic pulse sequences	1	1	2
Describe what makes something paramagnetic and determine effect on NMR constants		1	3
Describe purpose of shimming		2	2
Develop successful method to address problem		2	2
Describe the use of MRI to a lay audience		1	3
Answer the problem statement in a logical manner			4
Use clear, concise language		1	3
Illustrate data with graphs and tables		1	3

[a]Student lab reports ($N = 4$) were deconstructed into each of the learning objectives. Failing to meet the standard was not performing the task or completely missing the objective. Adequate achievement was providing answers without detail or explanation, but providing correct ideas. Excellent achievement was demonstrating the objective with proper argument and detail.

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weight, and if a student had failed to address a topic, s/he was required to redo the assignment before coming to lab. A combined, example student report is provided in the Supporting Information.

SUMMARY

This problem-based experiment on quality control of citrus fruit focused on NMR/MRI was designed and successfully implemented in an undergraduate upper-level chemical measurements course. Students were successfully able to construct content and procedural knowledge about NMR and MRI and defend their results in the context of a quality control scenario. Classroom-ready materials to teach and assess the experiment were improved slightly after implementation and address authentic student questions and concerns raised while carrying out the experiment. This lab is ready to be implemented in new settings, and the authors welcome information about its performance in other contexts.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00251.

Facilitation guide for the instructor (PDF, DOCX)
Problem statement (PDF, DOCX)
Student laboratory guide (pre-lab exercises, experiment guide, and report guide) (PDF, DOCX)
Report grading rubric (PDF, DOC)
Sample student report amd spectra collected by students and the authors (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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