

An Oil Spill in a Tube: An Accessible Approach for Teaching Environmental NMR Spectroscopy

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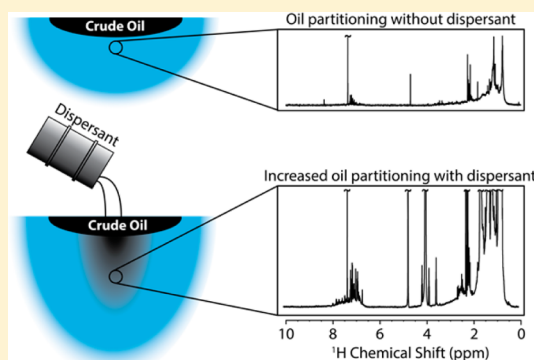
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Supporting Information

ABSTRACT: NMR spectroscopy has great potential as an instrumental method for environmental chemistry research and monitoring but may be underused in teaching laboratories because of its complexity and the level of expertise required in operating the instrument and interpreting data. This laboratory experiment introduces environmental NMR spectroscopy to upper-level undergraduate and graduate students in a simple and accessible manner. Students investigate the partitioning of crude oil components into water under various environmental conditions; assess the effects of agitation and dispersants on dissolution; and identify benzene, toluene, ethylbenzene, and xylene components through standard addition. Furthermore, electronic referencing is introduced as an effective method for quantification in complex unaltered samples.

KEYWORDS: Upper-Division Undergraduate, Graduate Education/Research, Environmental Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, NMR Spectroscopy, Micelles, Equilibrium, Qualitative Analysis, Quantitative Analysis



INTRODUCTION

NMR spectroscopy is one of the most powerful tools in modern research with unparalleled capabilities for the investigation of molecular structure and interactions in complex samples. NMR is growing into a versatile and powerful tool to study environmental systems and processes in their native states¹ and provides key molecular-level information that can be used to unravel large-scale environmental processes.² Despite its potential, NMR spectroscopy is still underutilized in environmental research in part because of a lack of hands-on exposure to the technique by students early in their careers in more traditional disciplines such as geology and soil science.^{1–5} Educational experiments using instruments that are widely applied in environmental research such as gas chromatography–mass spectrometry, ion chromatography, and atomic absorption spectrometry are abundant,^{6–10} as are upper-level NMR laboratories with an organic and inorganic focus.^{11–13} However, laboratories that cover NMR spectroscopy in an environmental context are lacking.

The experiment described herein was developed over three years and performed by over 40 graduate students with both chemistry and environmental science backgrounds. It is undertaken over two 4 h laboratory periods that follow an introductory session that covers the basics of ¹H NMR mixture analysis and structure elucidation.¹⁴ The approach taken assumes no prior NMR knowledge and facilitates accessibility for environmental program graduates. Techniques are intro-

duced in the context of an environmental oil spill and specifically address the action of dispersants and agitation on aqueous solubility of toxic benzene, toluene, ethylbenzene, and xylene (BTEX) components from crude oil.

The experiment covers a wide range of practical learning objectives, which include nonselective detection, nondestructive analysis, standard addition, serial dilution, compound identification, solvent-induced chemical shift perturbation, NMR water suppression, and advanced quantification using the electronic referencing technique known as electronic reference to access in vivo concentrations (ERETIC).¹⁵ The latter technique circumvents problems with internal standards, which often bind to environmental samples, and provides a rapid and accurate quantification approach for natural samples in their intact state. Chemistry students will learn about environmental concepts that are not traditionally taught, including advantages and disadvantages of using dispersants on oil spills, contamination of groundwater by BTEX components, BTEX toxicity, and various types of crude oil (sour/sweet, light/heavy, paraffinic/naphthenic). Since the laboratory assumes no previous NMR knowledge and is relatively simple for scientists with varying skill sets, it could be positioned as part of an analytical chemistry or environmental chemistry undergraduate or graduate course.

NMR SAMPLE PREPARATION AND DATA ACQUISITION

This experiment used crude oil number 2 from a commercially available kit of crude oil samples (ONTA Inc., Toronto, ON, Canada). The oil is a paraffinic crude oil from South Louisiana and has a light density of 0.839 g mL^{-1} at 15.56°C and 0.21% sulfur content (classified as a sweet crude oil). Several NMR tubes (labeled A–E) were prepared by students as follows: A, $500 \mu\text{L}$ of crude oil + 1 mL of D_2O (unshaken, Figure 1A); B,

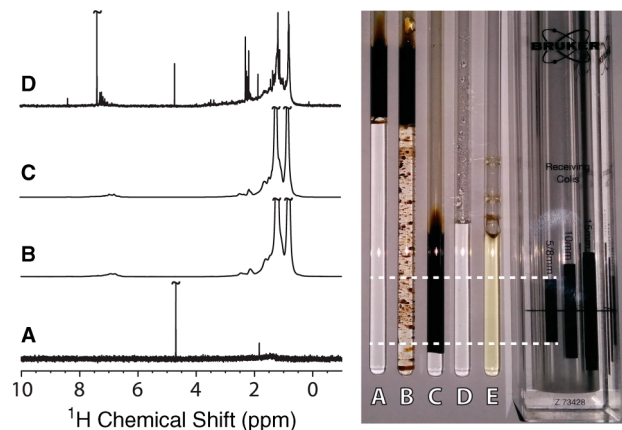


Figure 1. ^1H NMR spectra acquired for tubes A–D (left). Photograph of tubes A–E beside an NMR depth gauge (right). The first vertical black bar on the depth gauge and the associated dashed white lines indicate the position of the NMR coil and thus the portion of the sample being analyzed. Tube E is studied in detail in Figure 2 and the corresponding text. All NMR experiments were collected using identical conditions; the differing signal-to-noise between samples indicates the relative concentrations of oil components within the NMR coil in each case.

$500 \mu\text{L}$ of crude oil + 1 mL of D_2O (mixed by inverting the NMR tube approximately ten times, Figure 1B); C, $900 \mu\text{L}$ of crude oil + $100 \mu\text{L}$ of D_2O (unshaken, Figure 1C); D, $500 \mu\text{L}$ of crude oil + 1 mL of D_2O (vigorously shaken together in a scintillation vial, lower aqueous layer placed in NMR tube after

separation of layers, Figure 1D and Figure 2, upper trace); E, $500 \mu\text{L}$ of crude oil + 1 mL of 0.1 M sodium dodecyl sulfate (SDS) in D_2O (vigorously shaken together in a scintillation vial, lower aqueous layer transferred to NMR tube after separation of layers, Figure 2, lower trace).

Up to six more tubes identical to E were subsequently prepared with one of the following BTEX components added to each tube: benzene, ethylbenzene, toluene, *o*-xylene, *m*-xylene, or *p*-xylene. Through spectral comparison, it is possible to see which peaks increase in intensity and identify individual components unambiguously, even within an ultracomplex mixture such as crude oil. NMR data were collected using a Bruker Avance 500 MHz NMR spectrometer, and the acquisition parameters are described in detail in the Supporting Information. ERETIC was performed within TopSpin software (version 3.1, Bruker BioSpin) using a 2 mM sucrose solution for calibration of the electronic reference signal.¹⁶

HAZARDS

D_2O is slightly hazardous in case of ingestion. Benzene is flammable, toxic, causes skin and respiratory tract irritation, and is a known carcinogen. The remaining BTEX components (ethylbenzene, toluene, *o*-xylene, *m*-xylene, or *p*-xylene) are flammable, toxic, and cause skin and respiratory tract irritation. The crude oil samples may be harmful if absorbed through the skin and cause skin, eye, and respiratory tract irritation. SDS is flammable, harmful if swallowed, and causes skin irritation. Students should wash their hands thoroughly following the use of these chemicals. BTEX compounds and crude oil must be handled in a fume hood while wearing gloves, a laboratory coat, and adequate eye protection.

RESULTS AND DISCUSSION

Hydrophobicity and Mixing of Oil Components in Water

The ^1H NMR spectrum shown in Figure 1, panel A contains very few signals since few oil components in tube A partitioned into the aqueous layer due to their hydrophobicity. The lack of signals also demonstrates that experiments using two immiscible layers within an NMR tube can be easily designed

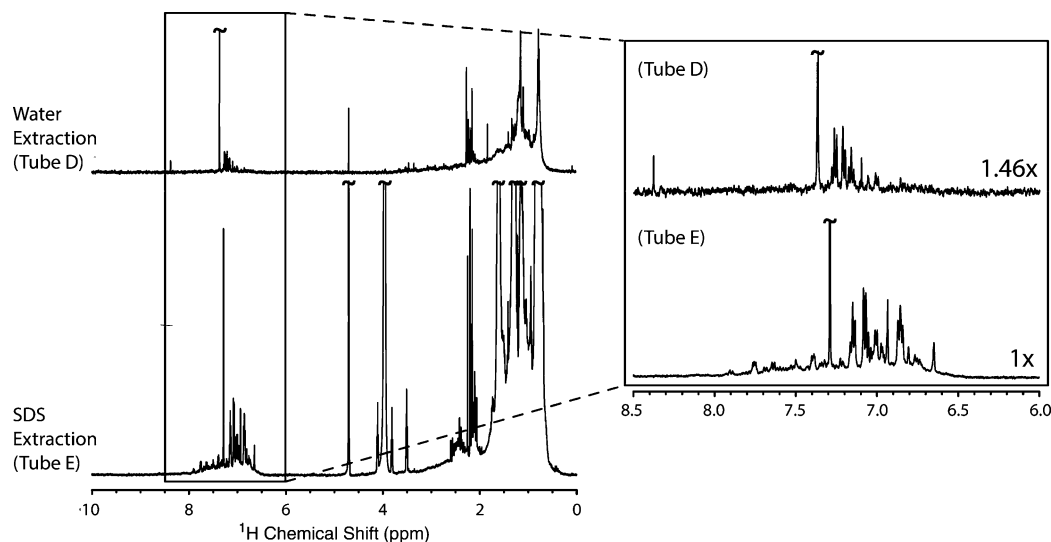


Figure 2. ^1H NMR spectra for samples in tubes D and E. The inset shows an expanded view of the aromatic proton region from 6–8.5 ppm for tubes D and E at 1.46 $\times$ and 1 $\times$ magnification, respectively.

such that signals from the upper layer are not detected if this layer is outside the NMR coil region (see Figure S1, Supporting Information). Tube agitation caused the aqueous and organic layers to mix and some components to be transferred from the oil to the aqueous phase; however, if students simply shake the NMR tube to mix the sample, the oil will stick to the sides of the glass (Figure 1, photo panel, tube B). By comparing the spectrum of pure oil (Figure 1C) to the spectrum after shaking (Figure 1B), it is apparent that signals in tube B arise from oil stuck to the side of the tube rather than discrete components dissolved from the oil phase. When the oil and water mixing is performed in a separate vial, the aqueous layer can be cleanly transferred without contamination from the bulk oil, and signals from oil components that truly dissolve into the aqueous phase can be observed (Figure 1D). In an oceanic oil spill, these compounds most likely leach into the water first. In the experimental NMR tube, shaking is being used to crudely mimic wave action and mixing in the environment.

Use of an Oil Dispersant

Ocean oil slicks are routinely broken up using dispersants. For example, an estimated 1.84 million gallons of dispersant was applied to the 2010 BP *Deepwater Horizon* oil spill in the Gulf of Mexico, with reports that indicated that oil toxicity increased by 52 times after dispersant application.¹⁷ In this experiment, SDS is used as a simple dispersant to mimic more complex proprietary mixtures that are used in practice. The surfactant properties of SDS increase the transfer of compounds from the oil to the aqueous layer, which can be seen in Figure 2, where the NMR spectrum contains many more peaks from oil components, particularly in the aromatic region. A reference spectrum of SDS is shown in Figure S2 of the Supporting Information.

The chemical shifts of the oil components were altered after the addition of SDS, which represents different proton environments. The SDS concentration of 0.1 M is well above the critical micelle concentration of 0.0082 M at 25 °C.¹⁸ Therefore, in the presence of SDS, most of the oil components are within micelles and aid dispersion into the water phase. This provides an excellent opportunity for students to learn about and understand micelle behavior first-hand. The question that many students raise is that “if NMR chemical shifts are so dependent on their solvated environment, how can accurate assignments be made?”

Standard Addition for Identification of BTEX Components

The simplest approach to provide accurate and unambiguous identification of exact structures within a complex mixture is through standard addition. BTEX components are present in most petroleum-based products, including oil and gasoline. They are distributed globally and represent a class of contaminants that are toxic to both humans and wildlife.¹⁹ Although at least one discrete signal for each BTEX component is apparent within crude oil (see Figure 3), one option is to focus on the standard addition of xylene compounds to avoid repetition. Figure 4 illustrates changes in the aliphatic resonance region after the addition of 25 nL of *o*-, *m*-, and *p*-xylene to each separate tube. The methyl group singlet for each isomer is clearly resolved, which permits assignment of matching peaks in the original spectrum (prespiking). The relatively simple resolution of xylenes provides occasion for students to compare results with those from gas chromatography–mass spectrometry, which may not always discriminate between isomers given their identical molecular weights.

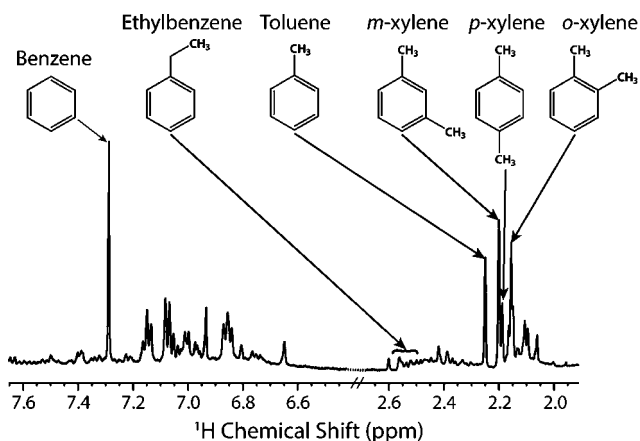


Figure 3. Assignment of the most prominent signal for each of the BTEX components. In the case of toluene and xylenes, the methyl signals are most indicative (3 protons per peak). Note that ethylbenzene was below detection limits in this particular oil sample. When ethylbenzene is abundant, the characteristic quartet from the CH₂ group dominates the region highlighted (2 protons). For benzene, the aromatic resonance must be used since this compound only produces a singlet (6 protons).

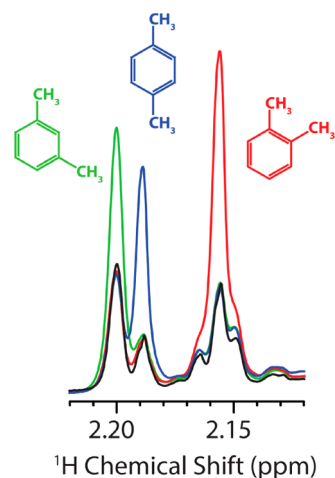


Figure 4. Expansion of the xylene benzylic region of the ¹H NMR spectrum of an oil–water mixture containing SDS both before (black trace) and after spiking with individual xylene isomers (colored traces). An increase in peak intensity upon spiking is used to positively identify each compound.

Quantification of BTEX Components

In addition to compound identification, standard addition is also commonly used to quantify components in NMR spectroscopy; however, since the components are immiscible with water and are volatile, accurate standard addition is challenging. A separate water-miscible internal standard might potentially be used, although if this compound interacts or partitions into the SDS micelles, it could influence quantification by affecting line-shape or causing precipitation. The take-home message for students is that when working with natural samples, it is ideal to perturb the system as little as possible. A novel technique (ERETIC) permits an external electronic reference peak to be added to a spectrum that has been calibrated to a known standard. The result is that a peak of known concentration can be artificially added to a spectrum,

permitting quantification, without physically adding anything to the tube.¹³

Figure 5 shows the ^1H NMR spectrum of tube E after the addition of a synthetic ERETIC peak. The added peak is

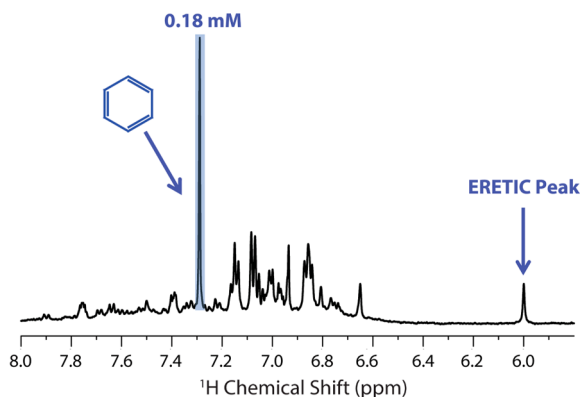


Figure 5. Expansion of the aromatic region of the ^1H NMR spectrum of an oil–water mixture containing SDS. An ERETIC reference peak was added at 6.00 ppm, which corresponds to 0.2 mM (one proton per peak). The calculated concentration for benzene in the sample is shown above the highlighted peak.

equivalent to a compound containing one proton of 0.2 mM concentration and essentially takes the place of an external standard. To quantify the concentration of BTEX components, students need to select the peak of interest (Figure 5 highlights the resonance of benzene) and supply the number of protons for the integrated peak (e.g., for benzene, the singlet represents all six protons in the aromatic ring). The software then calculates the concentration in mM and places the results as text above the peak as shown in Figure 5.

The number of protons per resonance must be known to calculate the concentration by NMR, and this information needs to be supplied to the ERETIC software. Students are referred to an excellent article by Wallace regarding NMR quantification.²⁰ In this laboratory, the concentrations of the BTEX components are of interest, and after assignment (achieved by standard addition), the multiplicity of each resonance is known. Students may wish to acquire the oil spectrum in triplicate in order to provide an estimation of error. If time is limited, it is possible to simply ask the same students or different students to repeat the manual integration and ERETIC calculations, permitting an experimental error to be reported for the analysis component of the laboratory report. Furthermore, as the synthetic ERETIC signal can be added to a spectrum that corresponds to any concentration, it serves as a useful tool to evaluate limits of detection and quantification. In this experiment, a signal-to-noise threshold of 3:1 is used to define a peak (i.e., limit of detection), and a signal-to-noise threshold of 10:1 permits peak quantification (i.e., limit of quantification). Students can then add peaks of varying concentration (they provide a concentration in mM and the software generates the corresponding peak) interactively into the experimental spectrum until the signal-to-noise falls below the threshold value. This provides an interesting and interactive way to gauge the limits of detection and quantification in a user-friendly manner. To extend the laboratory further, an optional “research” component based on hypothesis development and testing is included in the Supporting Information (laboratory handout Section 4).

CONCLUSIONS

This laboratory has been offered for three consecutive years as part of a graduate course that covers analytical instrumentation in environmental science and chemistry research. Considering the wide range of students and the need to educate environmental researchers about the potential of NMR spectroscopy, an emphasis was placed upon demonstrating techniques in an accessible manner that are relevant to real-world issues. Feedback from the students was very positive and indicated that they found the experiment highly enjoyable and productive (see section C, Supporting Information). Through this exercise, students learn that NMR is not simply a means to elucidate structures of pure compounds, but also a valuable nondestructive and nonselective tool to analyze complex mixtures with considerable potential for environmental analysis and monitoring. Students see first-hand how agitation and surfactant use can disperse toxic compounds into water bodies and how NMR can be used to study these processes. Furthermore, students learn how to identify and quantify species directly through the use of standard addition and electronic referencing.

ASSOCIATED CONTENT

Supporting Information

Laboratory handouts including student and demonstrator/teaching assistant instructions, assignment questions, and an optional research component as well as NMR experimental parameters and student feedback. This material is available via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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