EDULAB FOR STUDENTS: FOURIER 80

NMR Gives You Wings

Analysis of Red Bull Energy Drink

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Experiment Hashtag #: #NMRGivesYouWings #Educate2Resonate

Keywords:

Compound identification, DOSY, HMQC, Selective excitation

Target group:

Advanced Undergraduate or Graduate, General Chemistry, Analytical Chemistry, Food Chemistry

Objectives:

- Acquire, process and analyze basic 1D and 2D NMR spectra.
- Identify major components of Red Bull energy drink through fingerprint matching.
- Gain experience running specialized experiments (HMQC, DOSY, selective excitation).

Background of the Experiment:

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical tool, one which has mainly been taught to undergraduates as a technique for identification and quantification of various chemical compounds. While historically, NMR has mainly been used in academia and industry for structure elucidation in organic chemistry, recent advancements have expanded its applications into many areas including metabolomics, pharmaceutical, environmental, and food quality research.[1-4]

In the food sector, NMR can be used to identify unknowns and quantify various metabolites in foods such as fish oils, wine, juice, coffee, meat, and beer.[4–11] In food research, the most commonly used NMR techniques involve one dimensional and two dimensional techniques, proton (^{1}H) and carbon (^{13}C) NMR, nitrogen (^{15}N) and phosphorus (^{31}P) NMR.[4,10,12]

This investigation aims to help students understand the growing applications of NMR, beyond that of its traditional uses in organic chemistry. The experiments are designed to introduce students to 1D and 2D NMR techniques through hands-on analysis of Red Bull energy drink. Red Bull is a popular beverage that traces its origins to a Thai energy drink (referred to as Krating Daeng) which was then modified and sold globally.[13-15]

Although other energy drinks can be substituted for this lab exercise, Red Bull is recommended to stay consistent with previously published high-field data which is used to help guide pattern matching and identification.

Preparation and Prerequisite:

To perform this experiment, a properly installed and adjusted Fourier 80 system with TopSpin Software is required. In addition, a Sonicator (optional) and a 1000 µL micropipette should be available.

The Red Bull experiments should take approximately four hours to perform, an additional two hours for a report, and it is assumed that students have completed introductory concepts of 1D and 2D NMR and basics of spectral interpretation. The experiments are designed to be ideally completed in groups of 3-6 students. This investigation aims to demonstrate key NMR concepts, including interpretation of 1D ^{1}H, HMQC, DOSY and selective excitation spectra.

Experimental Setup:

- Red Bull energy drink
- 5 mm NMR tube and cap (1)
- 50 mL beaker and parafilm
- High-field Red Bull 1D and 2D data (found in Appendix I)
- Pulse programs: wet, hmqcetgp, ledbpgppr2s, selgpse

Sample Preparation:

1. Degas the Red Bull prior to use. This can be done by allowing an open can of Red Bull to stand in the fridge overnight in a 50 mL beaker then shaking the remaining carbonation out the day of use. In addition, sample can be sonicated for 2-3 hours if a sonicator is readily available.

2. Transfer ~600 µL to a 5 mm NMR tube and cap it.

Glossary

NMR:
Spectroscopic analytical technique based on radio frequency-induced transitions between energy levels that atomic nuclei adopt in an external magnetic field as a result of their own magnetic moment.

Selective excitation:
Process of selecting a specific peak or set of peaks in the spectrum and exciting only those spins. This is done to minimize the interference from other peaks and is useful because it allows you to focus on the signal from a specific molecule.

Water suppression:
A technique used in NMR to minimize the usually strong signal deriving from water. This is important because the water signal can interfere with the signal from other molecules you are interested in investigating, resulting in information loss.

DOSY:
DOSY in NMR is the process of measuring the diffusion of a molecule in a sample. Diffusion Ordered Spectroscopy is done by measuring the intensity of the signal at different positions in the sample to identify the diffusion coefficient of a molecule.

Experimental Procedure:

Insert sample in the spectrometer and determine the P1 and O1P (chemical shift of the water signal) of the sample. Record a 1D ^{1}H WET spectrum with 2048 scans, an HMQC with 128 scans, and a DOSY experiment with 8 scans. Lastly, run 2 selective excitation experiments with 32 scans; in the first, set the O1P value to that of the most downfield peak in the ethanol triplet, and in the second, set the O1P value to that of the most upfield peak of the propylene glycol doublet.

Data Processing:

All spectra should be processed using standard protocols, including baseline correction and phasing, and calibrated using the sucrose doublet at 5.4181 ppm. More information can be found here: https://pubs.acs.org/doi/10.1021/ed086p360

In the HMQC spectrum, the 1D ^{1}H WET spectrum shall be used as the external projection. Completely removing the water streak from the HMQC can be challenging; options include qfil or qpol (both topspin processing options), t1away (a topspin au) or scaling T_1 noise in commercial packages such as MestraNova. Identification of Red Bull components can be done by comparison with provided high field data. All corresponding 500 MHz data is provided.

More processing information can additionally be found in the version 001 Fourier EduLab student guide.

Notes:

Glossary

Abbreviations

NMR:
Nuclear Magnetic Resonance

DOSY:
Diffusion ordered spectroscopy

HMQC:
Heteronuclear single quantum coherence spectroscopy

P1:
Length of the ^{1}H excitation pulse

O1P:
O1 (or O1P for the value in ppm) is the carrier frequency used for the hard pulses

WET:
Water suppression enhanced through T1 effects

DS:
Dummy scans

NS:
Number of scans

Results & Discussion:

To complete the exercise, answer the following questions:

1. Identify and label sucrose, β-glucose, α-glucose, citric acid, niacin, and caffeine in the 1D ^{1}H WET spectrum. Discuss one advantage and one disadvantage of using 1D NMR for compound identification.

2. Taurine is not visible in the 1D ^{1}H WET spectrum as it overlap with the sugars, but can be observed in the HMQC spectrum. Using a figure, identify and label the taurine peaks, and explain the advantage of using HMQC NMR for compound identification.

3. What information does the DOSY experiment provide? Using a labelled figure, discuss how it can be used to differentiate between sucrose and the glucose anomers.

4. Using the 1D ^{1}H WET spectrum and the selective excitation results, explain how ethanol and propylene glycol can be differentiated from each other. Demonstrate with a labelled figure. (optional)

5. Optional Question: Discuss the water suppression method used and compare it to at least 3 other water suppression techniques from the literature.

Key Take Home Messages:

- Recent developments in NMR have expanded its applications beyond historical uses as an analytical tool for structural elucidation in organic chemistry.
- Typically, a combination of 1D and 2D NMR experiments are required to provide information to elucidate and identify components in food samples.
- The DOSY experiment enables differentiation between different sizes of molecules at similar chemical shifts.
- Selective excitation experiments can be used to selectively detect compounds that overlap in 1D spectra.

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Appendix I – High-field (500 MHz) spectra of Red Bull:

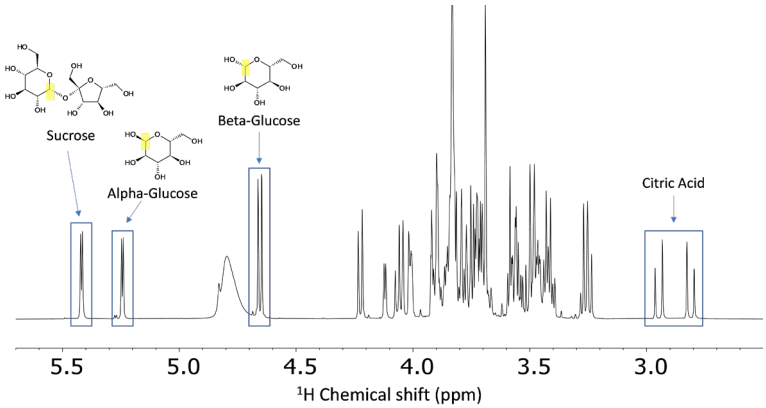

Figure A1. The 2.0-6.0 ppm range of the 500 MHz 1D ^{1}H NMR spectrum of Red Bull (for comparison purposes).

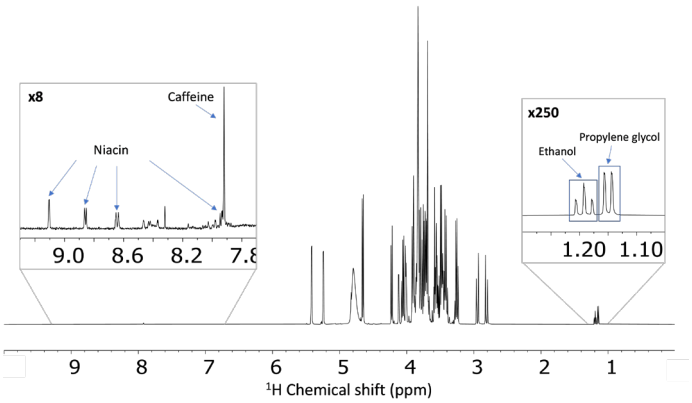

Figure A2. The 500 MHz 1D ^{1}H NMR spectrum of Red Bull, with insets depicting the aromatic region, as well as the ethanol and propylene glycol peaks.

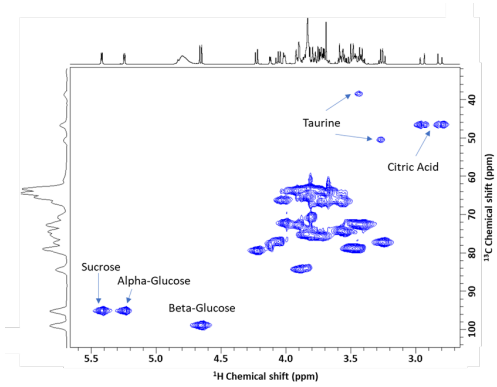

Figure A3. The 500 MHz HMQC spectrum of Red Bull.

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