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Introductory Chemistry: A Molar Relaxivity Experiment in the High School Classroom

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Abstract

Dotarem and Magnevist, two clinically available magnetic resonance imaging (MRI) contrast agents, were assessed in a high school science classroom with respect to which is the better contrast agent. Magnevist, the more efficacious contrast agent, has negative side effects because its gadolinium center can escape from its ligand. However, Dotarem, though a less efficacious contrast agent, is a safer drug choice. After the experiment, students are confronted with the FDA warning on Magnevist, which enabled a discussion of drug efficacy versus safety. We describe a laboratory experiment in which NMR spin lattice relaxation rate measurements are used to quantify the relaxivities of the active ingredients of Dotarem and Magnevist. The spin lattice relaxation rate gives the average amount of time it takes the excited nucleus to relax back to the original state. Students learn by constructing molar relaxivity curves based on inversion recovery data sets that Magnevist is more relaxive than Dotarem. This experiment is suitable for any analytical chemistry laboratory with access to NMR.

Which is the better MRI contrasting agent?

Keywords

High School/Introductory Chemistry; First-Year Undergraduate/General; Second-Year Undergraduate; Analytical Chemistry; Laboratory Instruction; Hands-On Learning/Manipulative; Applications of Chemistry; Drugs/Pharmaceutical; NMR Spectroscopy

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ASSOCIATED CONTENT

Supporting Information. Detailed synthetic procedures, spectra, and graphs. Instructions for students and instructors. This material is available via the Internet at <http://pubs.acs.org>.

Magnetic resonance imaging (MRI) is a ubiquitous imaging modality in modern medicine¹ and thus is a technique with which most high school students have at least passing familiarity. Many MRI images are acquired with the aid of a contrast agent drug. In this experiment we introduce the concepts of MRI and MRI contrast agents in a high school classroom by measuring the molar relaxivity curves for two clinically available MRI contrast agents. In the process of this experiment, students gain familiarity with magnetic relaxivity, exposure to simple data treatment, and basic analytical chemistry skills. At the conclusion of the lab, they learn that the more relaxive of the clinical agents is also the more toxic. This enables a practical discussion of drug efficacy versus drug safety and science in society.

This experiment requires access to an NMR spectrometer or relaximeter, which are tools that are not generally available to high school teachers. However, the U. S. National Science Foundation has prioritized both cyber-enabling of chemical instrumentation² and K-12 outreach³ to promote interaction between K-12 students and educators and NSF-sponsored investigators and facilities. It is in this spirit that we offer this as a high school lab experiment.⁴ Of course, it may be easily adopted for any introductory chemistry class or undergraduate analytic chemistry lab.

MRI and Contrast Agents

In many clinical applications, contrast of MRI images is enhanced by the use of one of several MRI contrast agents.⁵ A highly paramagnetic metal ion, Gd^{3+} in particular, which is chelated with an appropriate ligand, provides the basis of most of the current clinically available contrast agents in the United States.⁶ Because of its paramagnetism, Gd^{3+} promotes the rapid relaxation of nuclear spins that are excited in the MRI experiment. With an appropriate pulse sequence, this results in the amplification of MRI signals from aqueous regions that are in the proximity of the gadolinium agent.⁷

The basic principles of relaxation involved in MRI are those of nuclear magnetic resonance (NMR). The mechanism of contrast enhancement utilized by gadolinium-based agents involves accelerating the T_1 , the spin-lattice relaxation time constant,⁸ of the water surrounding the metal; thus these are said to be “ T_1 contrast agents”.⁹ The degree of efficacy of the contrast agent at any given concentration can thus be quantified by how short it can make the T_1 of the water in which it is dissolved. T_1 values can be easily measured on any NMR spectrometer. Therefore it is simple to assay an agent's efficacy using basic NMR tools.¹⁰

Clinical use of gadolinium-based MRI contrast agents is limited by the toxicity of the gadolinium(III) ion.¹¹ Because gadolinium(III) is an isostere for calcium(II),¹² gadolinium-based MRI contrast agents are associated with acute renal toxicity side effects, including nephrogenic systemic fibrosis (NSF).¹³ This toxicity can be mitigated by the design of the supporting chelating ligand in which the gadolinium ion is caged;^{6a} this is the basis for ongoing research in medicinal chemistry.¹⁴

Experimental Overview

In this experiment the dual issues of MRI contrast and drug efficacy versus drug safety are introduced. In the first part of the experiment, students prepare samples on which T_1 values can be measured for active ingredients in two MRI contrast agents, shown in Figure 1, Dotarem, $[\text{Gd}(\text{DOTA})]^-$ (1), and Magnevist, $[\text{Gd}(\text{DTPA})]^{2-}$ (2) where DOTA is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid salt and DTPA is diethylenetriaminepentaacetic acid salt. In the second part of the experiment, students process their T_1 data and find that $[\text{Gd}(\text{DTPA})]^{2-}$ (2), active ingredient in Magnevist, is a

more potent T_1 contrast agent. Upon making this finding, they are shown the FDA warning¹⁵ on Magnevist and taught why Dotarem is a safer clinical agent. This enables a discussion of safety versus efficacy of these agents.

Material and Instrumentation

For this experiment students used basic laboratory glassware, standard liquids 5 mm NMR tubes, coaxial insert tubes for 5 mm NMR tubes, disposable plastic syringes (or equivalent), disposable needles (or equivalent) with tips removed as desired, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, GdCl_3 , CaCO_3 , diethylenetriaminepentaacetic acid, Gd_2O_3 , D_2O , and deionized H_2O . Note that the water must be deionized; bottled drinking water is effective in our experience.

T_1 data were collected on a Varian 400-MR NMR spectrometer equipped with a 7600AS autosampler. T_1 data were processed with the VNMRJ 2.3 native T_1 processing regime and reported as measured (VNMRJ = Varian Nuclear Magnetic Resonance Java). Remote operation of the 400-MR spectrometer was enabled using VNC (Virtual Network Computing). The VNC server application native to Red Hat Linux was adequate to serve a VNC session from the instrument; the session is conveniently viewed with the freeware programs Chicken of the VNC for Mac (by Jason Harris) or RealVNC or TightVNC for Windows.

NMR Inversion Recovery (T_1)

NMR T_1 values are readily measured by an inversion-recovery pulse sequence that is available in any spectrometer's sequence library (Figure 2).⁸ Conceptually, the way this experiment works is that a 180° broadband pulse (p1) is delivered to the sample and then a variable-length relaxation delay (d2) follows. During the delay, some of the protons lose their 180° excitation, which is measured by a follow-up 90° pulse (pw). Tracking the integration of the resulting NMR signal as a function of the interpulse delay (d2) gives an exponential recovery curve with time constant T_1 .

Experimental Procedure

Stock solutions of gadolinium chelates can be prepared either by the instructors ahead of time or by the students as part of the experiment. Stock solutions of each active agent (1 mM) were provided to students for dilution to 0.25, 0.50, and 0.75 mM. Detailed synthetic procedures for the preparation of these solutions is presented in Supporting Information. Students were supplied with these standard 1 mM solutions and prepared dilutions of them to the respective final concentration and final volume of 1 mL with disposable 1 mL syringes with blunted needle tips. A portion (100 μL) of each of the samples was then transferred to an NMR coaxial insert tube, which was inserted into a standard 5 mm NMR tube containing 700 μL of D_2O .

The samples were remotely queued on a Varian 400 NMR spectrometer, and data were acquired in automation. An initial ^1H NMR spectrum followed by a T_1 inversion recovery experiment were acquired for each sample. Free induction decay (FIDs) signals were obtained and processed using the instrument's native VNMRJ software. T_1 data were presented to the students with fit error, as reported by VNMRJ. Students then plotted molar relaxivity curves, T_1^{-1} versus concentration, in the classroom using Microsoft Excel (see Supporting Information for group T_1 curves). The T_1 data from groups 1–4 were combined in a comparative molar relaxivity plot (Figure 3) to visualize the difference in the relaxivity between the two contrast agents. This experiment requires approximately 2 hours of class time, which was split over two days.

Hazards

Deuterium oxide, $[\text{Gd}(\text{DOTA})]^-$ (**1**), and $[\text{Gd}(\text{DTPA})]^{2-}$ (**2**) are hazardous if ingested. Gadolinium(III) chloride, gadolinium(III) oxide, DTPA, and DOTA are potential skin, eye, and respiratory irritants. Consult their respective material safety data sheets before use. See Supporting Information for chemical supplier information.

Results

T_1 values were acquired for $[\text{Gd}(\text{DOTA})]^-$ (**1**) and $[\text{Gd}(\text{DTPA})]^{2-}$ (**2**) (Table 1). We found that the average relaxivities were $r_1 = 2.6(2) \text{ mM}^{-1}\text{s}^{-1}$ and $r_1 = 4.3(4) \text{ mM}^{-1}\text{s}^{-1}$ for **1** and **2** respectively (Figure 3). With a higher r_1 (molar relaxivity) $[\text{Gd}(\text{DTPA})]^{2-}$ (**2**), active ingredient in Magnevist, was found to be the more relaxive contrast agent.

Discussion

The primary objective of high school science courses is to provide basic knowledge of the fundamental principles of science, mainly through the study of physics, biology, and chemistry. This often translates into a cursory investigation of each discipline. Students gravitate towards the acquisition and memorization of concepts and are bereft of the experience to appreciate the practical application of these ideas in real life. Thus the opportunity to engage in a research experiment enables students to exercise their newfound knowledge and helps foster retention of the information. In this particular experiment, students acquired empirical data to determine drug efficacy, which they then had to compare with actual results in the form of drug safety: in 2007 the FDA issued a boxed warning about renal toxicity associated with Magnevist and other gadolinium-based contrast agents.¹⁵ Dotarem, by contrast, is designed to avoid this toxicity problem.¹⁶

When faced with the notion that other factors need to be accounted for in data analysis, students then are forced to realize that science is not simply about learning formulas and plugging in numbers, but that its utility comes in assimilating knowledge with reality. Thus, this type of exercise was used to encourage students to recognize that beyond the basics taught in the classroom, there is real practical application of these concepts in our everyday lives.

The student experience in this exercise was assessed via student interviews. Through the interviews, it was found that the students were engaged and prompted to consider the broader implications of the subject they study all year in school. Although not all students grasped every detail, whether students gained an advanced understanding of MRI seemed a secondary issue. The primary purpose of this activity was to demonstrate an aspect of chemistry that is not immediately obvious to students and to encourage them to consider drug efficacy versus safety inherent in medicine and pharmacology. It was clear that the students enjoyed the lab and walked away with a new appreciation for the numerous applications of chemistry.

Conclusion

The procedures and data in this manuscript should enable instructors to use standard T_1 measurement techniques to demonstrate molar relaxivity measurements in an educational laboratory. Furthermore, use of the provided T_1 data could be the basis of an analytical problem set that demonstrates the process of calculating molar relaxivities. Ongoing educational research in our NMR facility includes the development of useful exercises to

teach undergraduate students both organic structure determination and basic analytical chemistry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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$[\text{Gd}(\text{DOTA})]^-$ anion

1

$[\text{Gd}(\text{DTPA})]^{2-}$ dianion

2

Figure 1.
Structures of the active agents in Dotarem (**1**) and Magnevist (**2**).

Figure 2. Varian's "T1 Measure" Inversion Recovery Pulse Sequence. Axes are transmit power versus time for the proton (Tx) and carbon (Dec) channels for the three portions of the pulse sequence, delay (A), pulse (B), and acquire (C). d1 = recycle delay (s); p1 = broadband inversion pulse width (μs); d2 = interpulse delay (ms); pw = detection pulse width, (μs); at = acquisition time (s).

Figure 3. Molar relaxivity curves of $[Gd(DOTA)]^-$ (1) (red, student groups 1 and 2) and $[Gd(DTPA)]^{2-}$ (2) (blue, student groups 3 and 4). Error bars describe fit errors in the T_1 data, not measurement errors that might have been introduced by the students.

Table 1Student Measured T_1 Values for Samples Prepared at Polytechnic School

Active Agent	[Gd] / mM	T_1^a / s
[Gd(DOTA)] ⁻ (1)	0.25	0.679(26)
	0.50	0.488(16)
	0.75	0.344(6)
	1.00	0.284(3)
	0.25	0.699(30)
	0.50	0.484(16)
	0.75	0.394(79)
	1.00	0.304(34)
[(Gd(DTPA))] ²⁻ (2)	0.25	0.364(9)
	0.50	0.288(3)
	0.75	* <i>b</i>
	1.00	0.157(1)
	0.25	0.339(3)
	0.50	0.291(3)
	0.75	0.205(3)
	1.00	0.181(1)

^a T_1 values and their standard fit errors generated by Varian's VNMRJ 2.3c software. Standard errors are represented in parentheses in msec.

^bThis sample did not conform to a T_1 fit due to an acquisition error.