

Divalent Metal Cation Assays Using EDTA and Benchtop Quantitative NMR



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INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA), sometimes used as its sodiated form, tetrasodium EDTA, is undoubtedly one of the most famous chelating agents in chemistry. As a hexadentate chelator, it can bind via two nitrogen donors, and four oxygen anions to encapsulate various metals. Typically, it is used to bind divalent metal cations (M^{2+}), and a variety of these types of complexes have been known for decades (Figure 1).

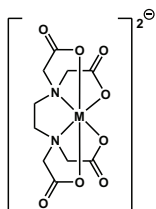


Figure 1. General structure of divalent metal cations encapsulated by EDTA. For the purposes of this study, $M = Zn, Mg, Ca,$ and Pb .

The quantification of metals is often performed using advanced analytical techniques such as inductively coupled plasma paired with optical emission spectroscopy (ICP-OES) or mass spectrometry (ICP-MS), meaning that undergraduate students are not typically introduced to these types of assays in their laboratory sessions. Alternatively, complexometric titration with either a visual indicator or an electrode have been used for many years, but the sensitivity of these techniques to parameters such as pH or temperature can add a layer of complexity to these analyses. Since it is well-known that the development of psychomotor skills is crucial for meaningful learning to occur,^{1,2} in this case in the use of analytical instrumentation, it is important for students to gain hands-on expertise in these areas.

Traditionally, high-field NMR has mostly been used for structural elucidation, reaction monitoring, and overall molecular characterization. However, quantitative NMR (qNMR) is also a powerful technique, as the integration area of each signal is directly proportional to the molar concentration of the species giving rise to that signal.³ As such, specific analytical reference standards are not required to gain quantitative information from an NMR spectrum. However, high-field NMR suffers from accessibility issues,



compounded by high upfront and recurring costs, the need for dedicated staff to maintain the instrument, and the requirements for liquid cryogenics to cool the superconducting magnet (*i.e.*, liquid nitrogen and liquid helium). Over the past 10 years, benchtop NMR has greatly increased the reach of this technique, enabling new uses across various underserved markets, including undergraduate laboratories.⁴⁻⁹ Here, we demonstrate the use of benchtop qNMR for performing assays on divalent metal cations using EDTA and demonstrate the usefulness of this approach by applying it to the quantification of calcium carbonate in a commercial product.

Experimental

The work performed herein is based on the publication by Diehl *et al.* in *Magnetic Resonance in Chemistry* and is adapted for use on a 60 MHz benchtop NMR instrument.¹⁰

For this study, the following chemicals were used as received and without further purification: MilliporeSigma: zinc(II) chloride ($\geq 98\%$), magnesium(II) sulfate ($\geq 99.5\%$), calcium(II) sulfate ($\geq 99.99\%$), lead(II) nitrate ($\geq 99.0\%$), cesium carbonate (99.9%), and ethylenediaminetetraacetic acid ($\geq 98.5\%$); Deutero GmbH: deuterium oxide (99.9%) and sodium 3-(trimethylsilyl)propionic-2,2,3,3- d_4 acid (99%). TUMS[®] Assorted Fruit Extra Strength antacid was purchased from a local pharmacy.

The following acquisition parameters were used for all analyses: spectral width, 24 ppm; number of points, 8192; scan delay, 1s; number of scans, 16; spectral center, 5 ppm; dummy scans, 0; pulse angle, 90°; gain: auto; acquisition time, 5.5 s.

The analysis of individual divalent metal cations was performed by first accurately preparing a stock solution of EDTA, by co-weighing the solids and dissolving them in the same volumetric flask: 50 mg/mL EDTA in D_2O , 2-3 mg/mL of TMS- d_4 , and 115 mg/mL of Cs_2CO_3 . These amounts are approximate, but the exact mass of EDTA must be recorded. Then, approximately 15 mg of $ZnCl_2$, $MgSO_4$, or $CaSO_4$ were accurately weighed in individual 1-dram vials and dissolved in exactly 0.6 mL of the stock solution using a calibrated micropipette. For $Pb(NO_3)_2$, approximately 35-40 mg of sample was accurately weighed in a 1-dram vial and dissolved in the same amount of stock solution. Then, each sample was transferred to a standard 5 mm NMR tube and placed in a 60 MHz benchtop NMR instrument for analysis.

The assays of the TUMS[®] antacid were performed by preparing a similar stock solution as above, but with an overall concentration of approximately six times higher for each component. Then, a single antacid tablet was accurately weighed, finely ground and

homogenized using a mortar and pestle, and ~50 mg were accurately weighed and dissolved in 0.6 mL of the concentrated stock solution. Prior to analysis, the samples were filtered into a standard 5 mm NMR tube using a ~1 cm plug of Celite[®], and an additional 0.1 mL of D_2O were added to wash the filtrate and make up for the solvent volume lost during filtration. A clear solution should be obtained. Then, the sample was placed in an instrument for analysis.

Analysis of $ZnCl_2$, $MgSO_4$, $CaSO_4$, and $Pb(NO_3)_2$

The 1H (60 MHz) benchtop NMR spectra of the Zn-, Mg-, Ca-, and Pb-EDTA samples are shown in Figure 2. While technically possible, the use of ^{67}Zn , ^{25}Mg , ^{43}Ca , and ^{207}Pb NMR is uncommon and typically reserved for specific applications or academic research, and more commonly used in solid-state NMR. The main reasons for this are due to the quadrupolar nature of these nuclides (nuclear spin $I = 5/2$ for $^{67}Zn/^{25}Mg$; $I = 7/2$ for ^{43}Ca) or their low natural abundance ($^{67}Zn = 4.1\%$; $^{43}Ca = 0.1\%$). While ^{207}Pb NMR is more accessible, with a nuclear spin $I = 1/2$ and a natural abundance of 22.1%, it is uncommon for users to tune to this nuclide for routine analyses.

The chelation of EDTA leads to a significant and measurable chemical shift difference in both the peaks arising from the $N-CH_2-COO-$ and $N-CH_2-CH_2-N$ protons compared to free EDTA. By analyzing a sample of the EDTA stock solution without any metal present, and by using the expected integration ratios of 2:1, the peaks can be assigned and integrated to obtain quantitative information about the metals. While the chelated Zn-EDTA complex exhibits notably different chemical shifts compared to free EDTA, allowing for the integration of individual signals, the Mg- and Ca-EDTA complexes give rise to broad and overlapping peaks, likely due to interconversion between the Δ and Λ conformers, as previously described.¹⁰⁻¹² In these systems, the acetate groups can switch positions, leading to a corresponding switch in proton positions, which can be expressed as: axial in plane \leftrightarrow equatorial out-of-plane, and axial out-of-plane \leftrightarrow equatorial in-plane. The rate of interconversion of these atropisomers¹³ is related to both the strengths of the metal-oxygen and metal-nitrogen bonds, in addition to the temperature of analysis. The coalescence of these enantiomeric signals, which are indistinguishable by NMR, can occur, leading to a collapse of the AB spin system into broad singlets. However, the chelated $N-CH_2-CH_2-N$ peaks remain sufficiently separated for integration and allows for the quantification of free EDTA via subtraction of those integration areas from those of the overlapping region. Finally, the Pb-EDTA spectrum reveals some $J_{207Pb,1H}$ coupling, as evidenced by the coupled signals with ~22% the intensity of the main signal, owing to the natural abundance of lead-207.

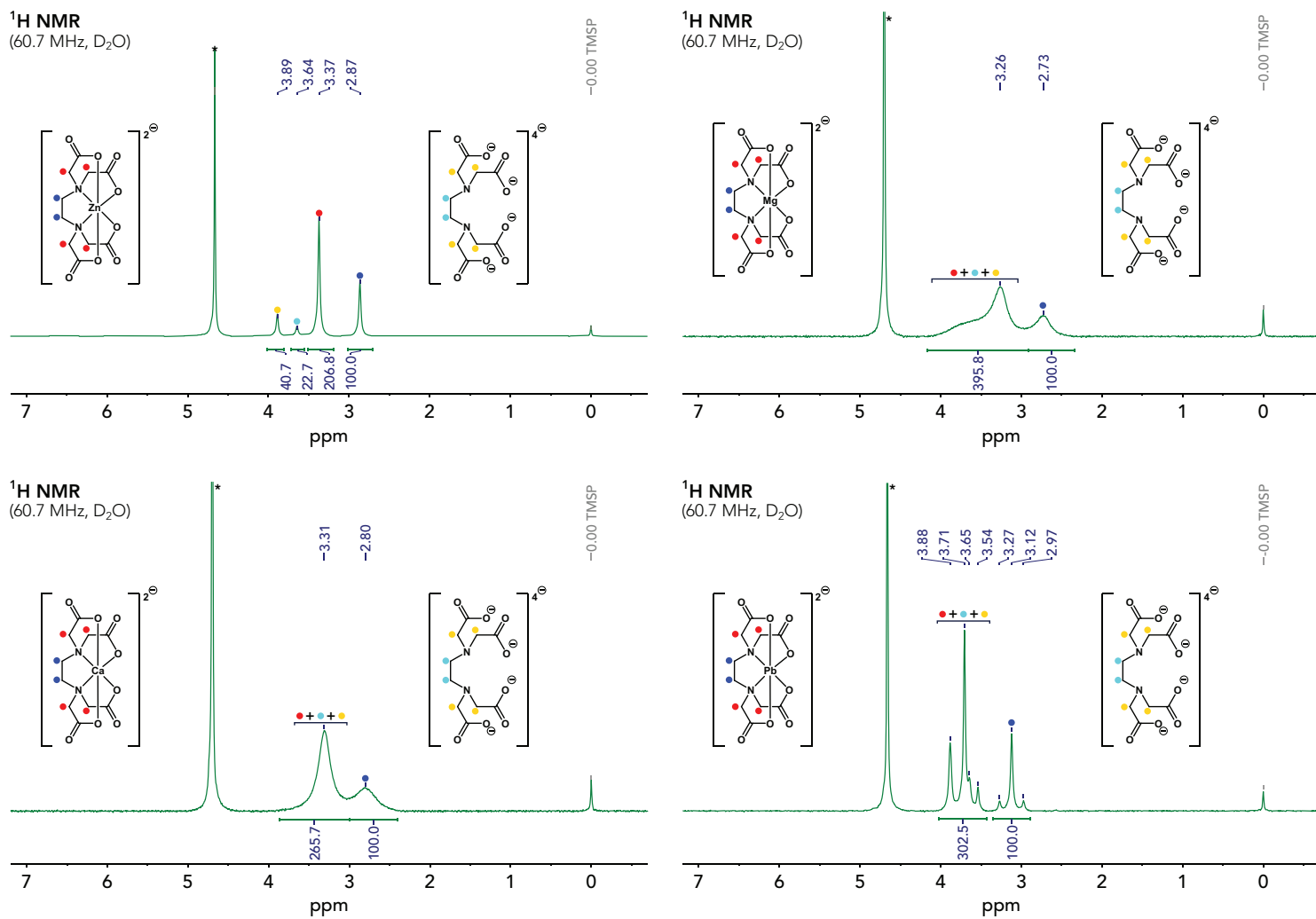


Figure 2. ^1H (60.7 MHz) NMR spectra of Zn-EDTA (top left), Mg-EDTA (top right), Ca-EDTA (bottom left), and Pb-EDTA (bottom right) in D_2O with TMSP added as a chemical shift reference. The pH is adjusted to ~ 7.2 using Cs_2CO_3 prior to complexation. The peaks arising from complexed and free EDTA are labelled. The asterisk represents the residual solvent peak for HOD.

The qNMR calculations were performed using the integration values included in Figure 2, and the results are presented in Table 1. The calculated amounts of metal salts were compared to the values obtained during weighing and agreed closely for all samples.

Analysis of CaCO_3 in an antacid tablet

To demonstrate the capabilities of this approach for the analysis of commercial samples, a bottle of TUMS[®] Assorted Fruit Extra Strength antacid was purchased from a local pharmacy and the calcium

content of a tablet was determined and compared to the value on the label (750 mg). The value indicated on the label must adhere to the monograph published by the United States Pharmacopeia, which is officially listed as Calcium Carbonate Tablets.¹⁴ The monograph states that the tablets must contain no less than 90.0% and no more than 110.0% of the labeled amount of calcium carbonate, which leads to an acceptable range of 675-825 mg for this product.

Table 1. Calculated metal salt content from ^1H (60 MHz) benchtop qNMR compared to the weighed values from the analytical balance.

Divalent Metal Cations		
	qNMR (mg)	Analytical Balance (mg)
ZnCl ₂	12.90	12.72
MgSO ₄	9.57	9.67
CaSO ₄	12.97	12.58
Pb(NO ₃) ₂	28.67	28.76

To determine the amount of calcium carbonate in an antacid tablet, a single tablet was first accurately weighed, finely ground, and homogenized using a mortar and pestle. Then, a sample was prepared in a similar fashion to the one used for the individual divalent metal cations, and the resulting suspension was filtered into an NMR tube prior to analysis. In total, three individual physical samples were prepared, and each sample was analyzed in triplicate. This was done to demonstrate the robustness of the method under repeatability and reproducibility conditions. A representative spectrum is shown in Figure 3 and the results of these assays are presented in Table 2. In all cases, the calculated CaCO₃ amounts from qNMR match those printed on the label very closely.

Table 2. Calculated CaCO₃ content in a single tablet antacid tablet, in addition to standard deviation (stdev) and relative standard deviation (RSD) values from triplicate assays. The amounts were determined using ^1H (60 MHz) benchtop qNMR.

Antacid Tablet				
CaCO ₃ Content (mg/tablet)				
	Average	Stdev	RSD	Label
Sample 1	749	0.71	0.09%	
Sample 2	736	1.30	0.18%	750 (675-825)
Sample 3	746	2.12	0.28%	

In total, each analysis takes approximately 2 minutes, and the sample preparation is minimal, only involving the preparation of an EDTA stock solution and dissolution of a metal salt or powdered tablet. These experiments offer excellent teaching tools to educate students on the concepts of chelation, qNMR, and the analysis of commercial products. In addition, it offers an opportunity for students to learn about the United States Pharmacopeia, among other similar organizations responsible for setting the standards across various areas such as the pharmaceutical industry. This provides them with additional knowledge about official monograph methods and can lead to discussions about regulatory and compliance testing, topics rarely discussed in the undergraduate curriculum.

Conclusions

The work presented herein provides a method for students to quantify divalent metal cations. Notably, EDTA is used to chelate various metals, allowing for an indirect method to quantify amounts of initial metal salts using ^1H qNMR. Additionally, a commercial antacid product was analyzed, and its calcium carbonate content was quantified and compared to the label value. Overall, this approach makes use of the powerful capabilities of benchtop NMR technology and can address many of the accessibility issues commonly encountered with traditional high-field NMR instruments.

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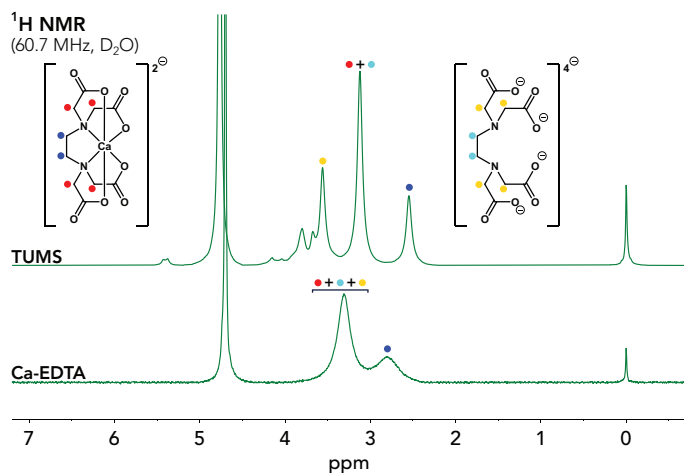


Figure 3. Stacked ^1H (60.7 MHz) NMR spectra of a dissolved antacid tablet (top) and Ca-EDTA (bottom) in D₂O with TMSF added as a chemical shift reference. The pH is adjusted to ~7.2 using Cs₂CO₃ prior to complexation. The peaks arising from complexed and free EDTA are labelled. The asterisk represents the residual solvent peak for HOD.

The ^1H (60 MHz) NMR spectrum of the dissolved and complexed antacid reveals the presence of the expected Ca-EDTA signals, in addition to various signals arising from the sucrose present in the tablet, as well as other minor components. Notably, the CH₂-COO- signals from free EDTA are shifted significantly to 3.56 ppm due to the increased pH and overlap with the signals from sucrose.¹⁰ As such, the signals at 2.55 ppm and 3.12 ppm were used to quantify the CaCO₃ in the tablet.



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