

Concentration Determination via Standard Addition

Incorporating NMR instrumentation into undergraduate laboratories increases students' understanding of fundamental and practical characterization techniques.

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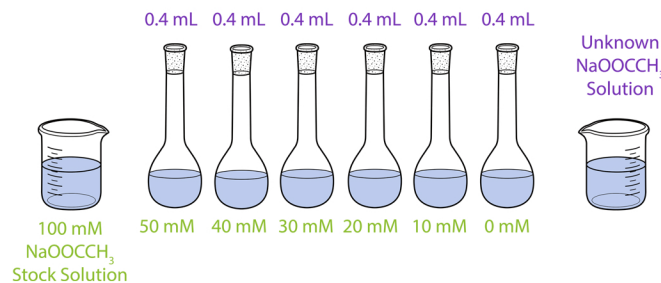
Quantitation by Standard Addition

Despite being the most commonly reported characterization technique^[1], NMR Spectroscopy is rarely included in undergraduate analytical laboratory curriculum. This is largely due to the availability of traditional NMR spectrometers for undergraduate use.

However, given the prevalence of this technique in synthetic organic and inorganic chemistry, we felt it important to introduce the students to an NMR spectrometer not only as a qualitative structure elucidation tool, but also as a quantitative instrument.

Proton NMR is an ideal technique for quantitation (sometimes referred to as qNMR) because, unlike most analytical techniques (e.g., MS or UV-Vis), every component has the same response factor and can be integrated directly to determine the amount of analyte in solution. This is most commonly exploited in qNMR by the addition of an ultra pure internal standard (IS). For this experiment, we have used a standard addition technique.

Standard addition is a quantitative method used to determine an analyte's concentration in a sample, typically where there are matrix effects that hinder accurate determination.^[1] A series of samples are prepared with the same amount of unknown solution and varying amount of a standard solution containing the analyte in known concentration.



Sample Preparation:

- 1) In small groups obtain a sodium acetate (NaOOCCH₃) solution of an unknown concentration from your TA.
- 2) In a volumetric flask prepare 5 mL of a 100 mM stock solution of NaOOCCH₃ in D₂O.
- 3) Pipette 0.400 mL of unknown into six 1 mL volumetric flasks.
- 4) Calculate the volume of 100 mM stock NaOOCCH₃ solution that must be added to each flask to make flasks at 0, 10, 20, 30, 40, 50 mM standard concentration. For example, in the 50 mM sample, use 1 mL x (50mM/100mM) = 0.5 mL.
- 5) Pipette appropriate amount of stock into each flask.
- 6) Dilute each to the mark with D₂O.
- 7) Transfer 0.5 mL from each volumetric flask to an NMR tube. Label.

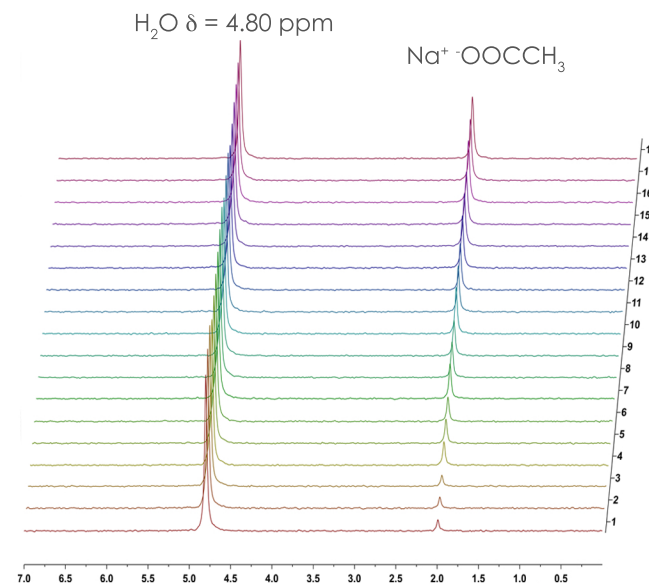
Data Acquisition:

- 8) Collect ¹H NMR data for each

spectral width = 12 ppm	#scans = 16
#points = 2k	scan delay = 40 sec
pulse width = 20.0 μsec	receiver gain = 25 dB
- 9) Save the data, be sure to include the date, your group name, and the known standard concentration in the tube you're measuring (e.g., YYYYMMDD_group1_40mM). Export to a network folder.
- 10) Using MNova, process your NMR data. Be sure to phase accurately, baseline correct & reference the water peak to 4.80 ppm.
- 11) Using MNova's advanced data fitting feature to create an integral graph.
- 12) Record the absolute integration of each spectrum in the range of δ = 1.7 - 2.3 ppm and tabulate integration vs. concentration.
- 13) Share the data within your lab section.
- 14) Determine your lab sections average & standard deviation.
- 15) Use standard addition equations and extrapolation to determine the concentration of the NaOOCCH₃ unknown.

Example of 60 MHz ¹H NMR Data:

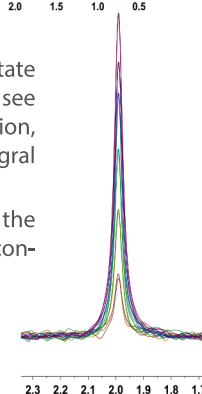
The example data is shown for 3 groups:



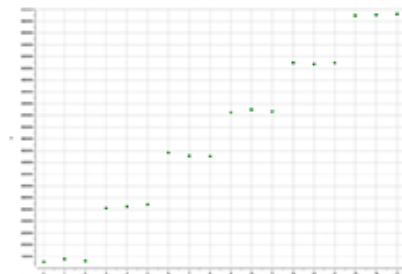
If we zoom in on the sodium acetate region in superimposed spectra, we see that depending upon the concentration, the absolute intensity of each integral changes.

The observed signal (I_{x+S}) is due to the unknown $[x]$ and the standard $[S]$ concentrations.

$$\left(\frac{[x]_i}{[S] + [x]} \right) = \left(\frac{I_x}{I_{x+S}} \right)$$



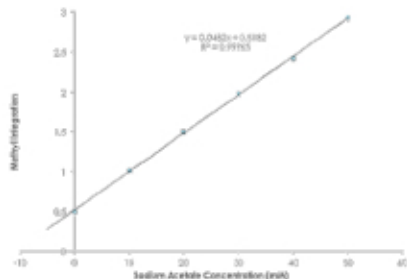
Using the MNova, we can create an integral graph that plots absolute integration vs. the spectra. This data is tabulated below for three groups.



Tabulated Results & Data Treatment:

Concentration of Standard (mM)	Group 1	Group 2	Group 3	Average	Standard Deviation
0	0.51	0.50	0.49	0.50	0.01
10	1.00	1.02	1.02	1.01	0.01
20	1.46	1.52	1.51	1.50	0.03
30	1.98	1.98	1.97	1.98	0.01
40	2.43	2.44	2.40	2.42	0.02
50	2.94	2.93	2.93	2.93	0.00

Plot your group's data vs. the concentration of sodium acetate standard added to each flask.



The equation of the line shows the resultant methyl integration (Int_{Me}) vs. standard concentration (V_s). The x-intercept gives unknown concentration.

$$y = mx + b$$

$$Int_{Me} = V_s \left(\frac{kC_s}{V_T} \right) + V_x \left(\frac{kC_x}{V_T} \right)$$

$$\text{where } m = \left(\frac{kC_s}{V_T} \right)$$

$$b = V_x \left(\frac{kC_x}{V_T} \right)$$

C_s = conc of std stock

C_x = unknown concentration

V_T = total sample volume

V_x = volume of standard added

V_s = volume of unknown added

k = response factor

$$\text{then, } C_x = \left(\frac{b}{m} \right) \left(\frac{C_s}{V_s} \right)$$

$$\text{and the concentration in original is } C_{2x} = \left(\frac{C_x V_T}{V_s} \right)$$

Calculate the apparent C_x for each group to determine the unknown concentration of the solution.

Assuming that the unknown concentration was initially 23 mM determine the relative error for each data set.

$$\text{relative error} = \left(\frac{\Delta x}{x} \right)$$

	Group 1	Group 2	Group 3	Average
C_x (from b/ m)	10.5	11.1	10.8	10.8
C_{2x} (mMol)	26	28	27	27
% Relative error	10	16	12	11

Discussion:

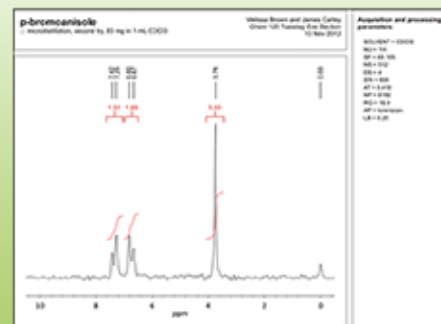
- 1) What was the concentration of unknown in the NMR tube?
- 2) What concentration of unknown in the original solution?
- 3) Why did we use the method of standard addition?
- 4) Why is the calibration curve linear?
- 5) We used an algebraic method to determine the x-intercept. How else could this be done?
- 6) What sources of error were introduced into the experiment?
- 7) What different procedure steps could be taken to reduce experimental error?
- 8) What other methods could have been used to determine the concentration of the unknown acetate concentration? Name at least two.
- 9) Would you be concerned if the solution was turbid? Would this affect the accuracy? Please explain thoroughly.
- 10) If you wanted to know the concentration of malonyl-coA in solution, detail a standard addition procedure that you would use to determine it's concentration.

References:

- 1) Harris, D. C. 'Quantitative Chemical Analysis', 5th Ed.; W.H. Freeman and Company: New York, 1999
- 2) Rajabzadeh, M.; J. Chem. Educ. 2012, 89, 1454
- 3) Bruce, G. R.; Gill, P. S. J. Chem. Educ. 1999, 76, 805
- 4) Bharti, S. L.; Roy, R. T. Anal. Chem. 2012, 35, 5

Data Accessibility:

NMReady outputs to a networked drive and has a print option. Students can process and print in third party software, like MNova™, or use the NMReady directly. An example of data to be incorporated into a lab report processed and printed directly from the NMReady is presented below:



For additional ideas of how to incorporate the NMReady™ benchtop spectrometer into undergraduate laboratories please see:

- 1) Aldol Condensation
- 2) Synthesis of Biodiesel & Reaction Monitoring
- 3) Determination of Acid Dissociation Constants

available at:

www.nanalysis.com/experiments.html