

Quantifying the Product Distribution of a Chemical Reaction by ^1H NMR Spectroscopy: A Cooperative Learning Approach for the Undergraduate Organic Chemistry Laboratory

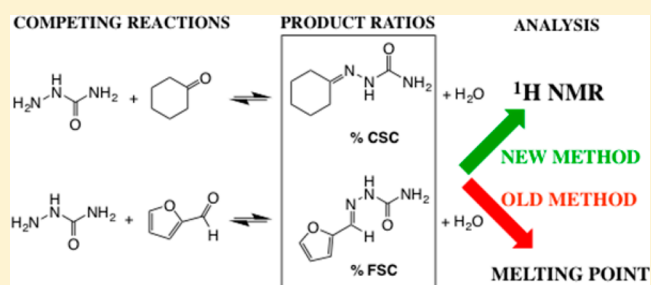
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S Supporting Information

ABSTRACT: This enhancement of a classic laboratory experiment investigates the kinetic and thermodynamic control of a reaction utilizing ^1H NMR spectroscopy instead of melting point analysis to determine the product distribution. In this experiment, semicarbazide is reacted with cyclohexanone and 2-furaldehyde under varying buffer and temperature conditions to yield semicarbazone derivative products. After ^1H NMR analysis of the products, students compile the class data in a cooperative setting facilitated by graduate teaching assistants. As a result, students are able to analyze a larger data set in this cooperative learning environment in order to describe the product distribution of the reaction on the basis of trends as opposed to using a single data set. The application of ^1H NMR spectroscopy to determine the product distribution of the reaction introduces a modern approach to study a well-established example of the kinetic and thermodynamic control of a reaction.

KEYWORDS: Second-Year Undergraduate, Laboratory Instruction, Organic Chemistry, Collaborative/Cooperative Learning, Inquiry-Based/Discovery Learning, Kinetics, Thermodynamics, NMR Spectroscopy



INTRODUCTION

Incorporating modern instruments into the undergraduate organic chemistry teaching laboratories is a pedagogical practical topic and a laboratory experience curriculum topic in the American Chemical Society (ACS) guidelines for Bachelor's degree programs.^{1,2} Even courses with large enrollments, 200–400 students per semester, are responsible for exposing students to the many ways that instruments can be used to analyze organic compounds.³ In this experiment, students are introduced to the application of ^1H NMR spectroscopy for the quantitative analysis of organic reaction products. There are several examples in the literature about the use of quantitative ^1H NMR spectroscopy in teaching laboratories in various contexts;^{4–7} however, this experiment focuses on kinetics and thermodynamics, which are conceptually challenging topics for many students.⁸

A recent survey showed that, of the most common topics covered in organic chemistry teaching laboratories, only 50% of respondents covered kinetics, 38% covered solvent effects, and 22% covered thermodynamics.⁹ These core concepts of organic chemistry, while not commonly covered in the teaching laboratory, show up in the organic chemistry course, advanced chemistry courses, and the Medical College Admission Test (MCAT) and are essential for students entering industry or pursuing graduate studies in chemistry.¹⁰ An experiment by Gilbert and Martin¹¹ that explored the question of thermody-

amic versus kinetic control of a competing reaction provided the foundation for this updated experiment.

In Gilbert and Martin's experiment, the products of a semicarbazide reaction between two competing carbonyl compounds were identified on the basis of melting points. The product ratio of each compound, cyclohexanone semicarbazone (CSC) and 2-furaldehyde semicarbazone (FSC), was based on the deviation from the pure melting point of each compound (Table 1). To quantitatively determine the product

Table 1. Structures and Melting Points of Possible Products

Structure	Name	Melting Point (°C)
	cyclohexanone semicarbazone (CSC)	165–167 ^a
	2-furaldehyde semicarbazone (FSC)	197–198 ^b

^aSee refs 12 and 13. ^bSee refs 14 and 15.

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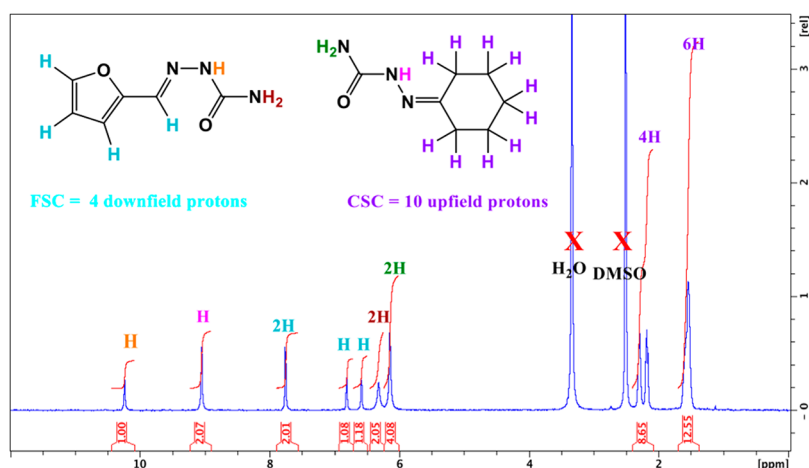


Figure 1. ^1H NMR spectrum of a prepared 1:2 mole ratio of FSC to CSC in $\text{DMSO-}d_6$.

ratio in these reactions, the modern application of ^1H NMR spectroscopy was implemented to update the original experiment. Applying this method of analysis reinforced the key properties of chemical shift and integration in ^1H NMR spectroscopy while introducing the concept of mixture analysis through comparative integration. This analysis utilized ^1H NMR spectroscopy beyond the usual structure determination methodology found in the undergraduate laboratory. Additionally, the sensitivity of the ^1H NMR method afforded experimental conditions appropriate for the microscale setting in order to reduce the amount of hazardous waste generated.¹⁶ Finally, two pedagogical goals for the updated experiment were accessed. One goal was to apply a cooperative and collaborative learning approach, proven to be effective in chemical education,^{17–20} to analyze the NMR data for identification and quantification of CSC and FSC. The second pedagogical goal was to use the large data set of student-pooled NMR results to assess the kinetic versus thermodynamic control of the reaction under study.

EXPERIMENTAL PROCEDURES

Materials and Equipment

A detailed procedure for the experiment, including all of the student handouts, is given in the [Supporting Information](#). The procedure includes the extended analysis that was completed but not related to the ^1H NMR analysis.

All of the reagents were purchased from Fisher Scientific or Sigma-Aldrich. ^1H NMR spectra were collected on a Bruker Avance 300 MHz spectrometer with an autosampler.

Lab Implementation

Each student completes one of the A OR B experiments and completes one of the C experiments.

Part A. A stock solution of semicarbazide hydrochloride (0.36 M) and dibasic potassium phosphate (0.46 M) is prepared in advance. An equimolar mixture of cyclohexanone and 2-furaldehyde in 95% ethanol is combined with the stock solution of the phosphate buffer under the following conditions: A1, 0–2 °C for 3–5 min; A2, 23 °C (room temperature) for 5 min followed by cooling in an ice bath for 5 min; A3, 80–85 °C for 10–15 min followed by cooling in an ice bath for 5 min.

Part B. A stock solution of semicarbazide hydrochloride (0.36 M) and sodium bicarbonate (0.95 M) is prepared in

advance. An equimolar mixture of cyclohexanone and 2-furaldehyde in 95% ethanol is combined with the stock solution of the bicarbonate buffer under the following conditions: B1, 23 °C (room temperature) for 5 min followed by cooling in an ice bath for 5 min; B2, 80–85 °C for 10–15 min followed by cooling in an ice bath for 5 min.

Samples for NMR analysis from parts A and B were prepared in dimethyl sulfoxide- d_6 ($\text{DMSO-}d_6$).

Part C. Since the two procedures below do not result in mixtures, the NMR method of analysis was not utilized.

Cyclohexanone semicarbazone and 2-furaldehyde semicarbazone are synthesized in advance following the method in ref 11.

C1: Cyclohexanone semicarbazone and 2-furaldehyde are mixed in 95% ethanol and water with gentle heating to produce a homogeneous solution. The mixture is cooled in an ice bath, and the resulting solid is analyzed by melting point.

C2: The same procedure as C1 except the two compounds are 2-furaldehyde semicarbazone and cyclohexanone.

HAZARDS

Cyclohexanone and 2-furaldehyde (freshly distilled) are irritating to the skin and flammable. Semicarbazide hydrochloride is irritating to the skin and toxic if ingested. Cyclohexanone semicarbazone and 2-furaldehyde semicarbazone are toxic if ingested. Hands should be washed thoroughly after handling these chemicals.

$\text{DMSO-}d_6$ is readily absorbed through the skin. Contact with skin should be avoided during preparation of NMR samples using $\text{DMSO-}d_6$. Standard and appropriate personal protective equipment (e.g., chemical splash goggles, lab apron or coat, long pants, and closed-toe shoes) is required for conducting the experiment. Instructors should consult the Safety Data Sheet for the products and the buffer components prior to beginning the experiment.

RESULTS AND DISCUSSION

Gilbert and Martin's general procedure was modified to use microscale-quantity reactions and to include more laboratory techniques in the analysis of the reaction products (see the [Supporting Information](#)). As in the original experiment, the reaction was performed at two or three different temperatures and with two different buffers (in parts A1–A3, phosphate buffer, pH 6; in parts B1–B2, bicarbonate buffer, pH 7). The

experiment was completed over the course of two laboratory periods.

In the first four-hour lab period, each student was assigned one part from A1–A3 or B1–B2. The ^1H NMR samples were prepared for the assigned reaction from part A or B. The first week of the experiment was timed so that students could complete the reactions and begin analysis of the products.

In the second four-hour lab period, students received their ^1H NMR spectra and determined the percentage of each reaction product in their samples. A worksheet (see the [Supporting Information](#)) was provided to the students, which was used to determine the percent composition of each product. The worksheets and the actual NMR spectra were collected from students at the conclusion of the laboratory. Students shared their data by entering their values into a spreadsheet on a computer located in the laboratory, and the class data were later posted on the university's course management Web site.

Figure 1 is the ^1H NMR spectrum of a prepared 1:2 mole ratio of FSC to CSC. Both CSC and FSC contain distinctive ^1H NMR signals that are easily distinguished from each other. Although both compounds contain NH and NH_2 groups, these exchangeable protons were avoided for quantification purposes. CSC contains 10 aliphatic protons of the cyclohexyl ring resonating upfield as three distinct peaks at $\delta = 2.4\text{--}1.4$ ppm. FSC contains four aromatic protons resonating downfield as three distinct peaks at $\delta = 6.5\text{--}8$ ppm. The selection of these signals reinforced the important concept of chemical shift differences between protons on sp^2 - and sp^3 -hybridized carbons. Analysis of the mole ratio first requires normalization of the integration of protons from each product. The CSC normalized proton integration is calculated by dividing the sum of the integrals of the upfield protons at $\delta = 2.4\text{--}1.4$ ppm by 10. Similarly, the FSC normalized proton integration is calculated by dividing the sum of the integrals of the downfield protons at $\delta = 6.5\text{--}8$ ppm by 4. The mole ratio of each product is easily determined by dividing each normalized proton integral by the sum of the two normalized proton integral values.

Table 2 shows the average mole percentage values calculated from the ^1H NMR spectra obtained by approximately 600 students enrolled in the second-semester organic chemistry laboratory during the 2010, 2011, and 2014 academic years. Using the data from academic year 2014 as an example, the lower-temperature reactions (parts A1, A2, and B1), regardless of the pH of the reaction, showed the major product to be CSC, the kinetic product, with mole percentages of $98 \pm 8\%$, $85 \pm 26\%$, and $95 \pm 15\%$, respectively. The higher-temperature reactions (parts A3 and B2), regardless of the pH of the reaction, showed the major product of the reaction to be FSC, the thermodynamic product, with mole percentages of $98 \pm 6\%$ and $76 \pm 30\%$, respectively. The data for all years confirmed an increase in the thermodynamic product with increasing temperature. The large standard deviations for parts A2 and B2, which were consistently observed for the three years studied, may reflect an enhanced sensitivity to the reaction time under those conditions. Samples of student experimental ^1H NMR spectra for parts A1 through B2 are shown in Figure 2. Parts A1, A3, and B1 typically yielded ^1H NMR spectra with a major product that was nearly 100% CSC or FSC, while parts A2 and B2 showed the greatest variability (Figure 2). In the latter case, the NMR analysis indicated to students that both

Table 2. Average Mole Percentage Values with Standard Deviations Calculated from ^1H NMR Spectra for Assigned Parts

Year	Assigned Part ^a	% CSC	% FSC	Student Replicates
2014	A1, $T \approx 0$ °C	98 ± 8	2 ± 8	40
	A2, $T \approx 23$ °C	85 ± 26	15 ± 26	41
	A3, $T \approx 80$ °C	2 ± 6	98 ± 6	39
	B1, $T \approx 23$ °C	95 ± 15	5 ± 15	46
	B2, $T \approx 80$ °C	24 ± 30	76 ± 30	35
2011	A1, $T \approx 0$ °C	99 ± 4	1 ± 4	57
	A2, $T \approx 23$ °C	89 ± 16	11 ± 16	50
	A3, $T \approx 80$ °C	1 ± 6	99 ± 6	44
	B1, $T \approx 23$ °C	100 ± 0	0 ± 0	42
	B2, $T \approx 80$ °C	46 ± 37	54 ± 37	41
2010	A1, $T \approx 0$ °C	88 ± 21	12 ± 21	41
	A2, $T \approx 23$ °C	51 ± 34	50 ± 34	39
	A3, $T \approx 80$ °C	10 ± 28	90 ± 28	35
	B1, $T \approx 23$ °C	97 ± 15	3 ± 15	42
	B2, $T \approx 80$ °C	34 ± 32	66 ± 32	41

^aPart A contained a phosphate buffer (pH 6), and part B contained a bicarbonate buffer (pH 7).

CSC and FSC could be formed under a single set of reaction conditions.

This experiment provided the opportunity to incorporate cooperative learning and shared discussion among students with teaching assistants as discussion facilitators in the laboratory.^{17,19,21} Upon completion of the experiment and return of ^1H NMR spectra, students worked together to calculate mole percentages of the products. Graduate teaching assistants worked closely with students to make sure their data were interpreted correctly. Reporting of class data enabled students to observe the variability of an experiment performed by hundreds of students and build confidence in their own experimental outcomes by having a chance to compare their findings to a large pool of data.^{22,23} In addition, the cooperative learning environment supported a platform for enhanced understanding of the shared data by engaging students in discussions of the product distribution of the reactions. Students were highly successful in using the NMR results to identify and quantify FSC and CSC, thereby achieving the first pedagogical goal of the updated experiment.

In the laboratory report, students were expected to explain and justify their identification of the thermodynamic and kinetic products of the reaction. The participation of the students in the cooperative learning environment in the laboratory was the foundation to prepare students to complete these questions independently in the report. In addition, the inquiry-based pedagogy of this experiment necessitated that students formulate the identities of the two products as the thermodynamic or kinetic product on the basis of their experimental evidence. Grading for these types of questions was focused on the ability of the students to argue and support their results. The definitive evidence of product formation obtained by ^1H NMR analysis of the reaction mixture provided a modern approach for determining the mole percentage and predicting the dominant product of the reaction rather than relying on melting point extrapolations, which can vary greatly with moisture content. Analysis of the 2014 laboratory reports support the achievement of the second pedagogical goal. Using the pooled NMR results, >99% of the class could correctly identify the kinetic (CSC) and thermodynamic (FSC) products

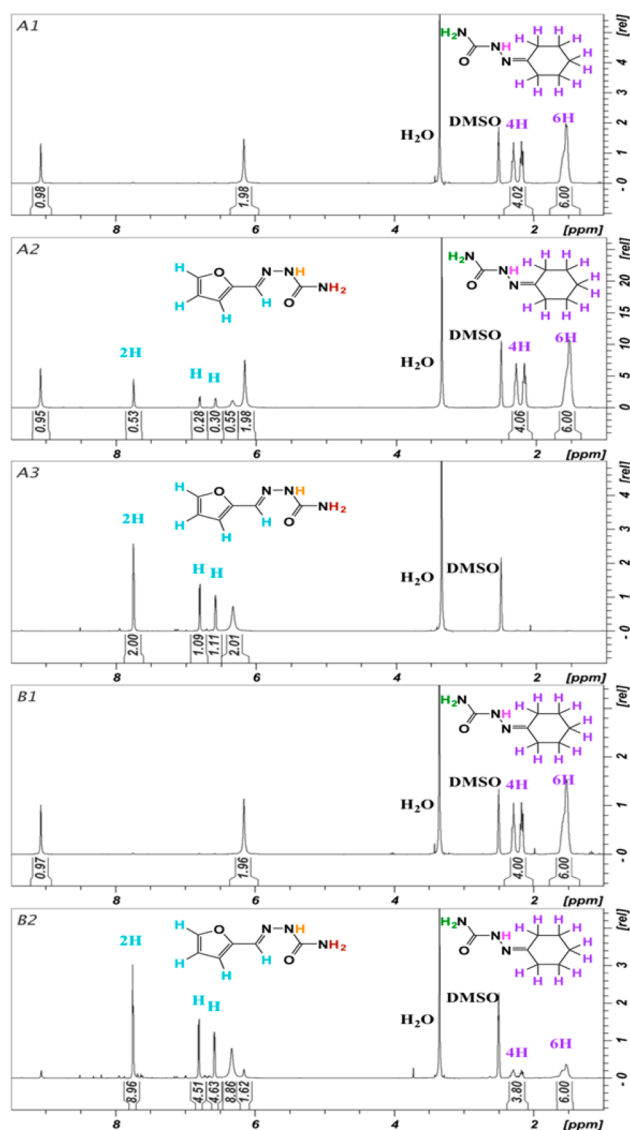


Figure 2. Samples of student experimental ^1H NMR spectra for parts A1–B2. The product distributions calculated from the ^1H NMR data shown were as follows: part A1, 100% CSC; part A2, 78.0% CSC and 22.0% FSC; part A3, 100% FSC; part B1, 100% CSC; part B2, 82.0% FSC and 18.0% CSC.

and >71% could explain the effect of pH on the dominant pathway of the reaction to form CSC or FSC.

Organic teaching laboratories often have students work alone and make conclusions on the basis of the outcome of a single experiment or single set of data. However, this practice is not representative of research at the graduate level or science in an industrial setting. Incorporating techniques that provide structural evidence of the molecules under investigation is necessary for conclusive identification of the products. This experiment was designed to update an experiment that covered an important concept in the undergraduate organic chemistry course, kinetic versus thermodynamic control, but lacked current pedagogical components as outlined by the ACS.^{1,2} First, students collaborated with one another, which reflected a common experience of actual practitioners of science. Second, students learned how to interpret trends in large data sets. Third, students were able to use physical data and structural evidence to support their experimental results.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.6b00601.

Detailed procedure for the experiment, classroom worksheet for calculating and reporting the mole percentage from the ^1H NMR spectrum, laboratory report template for the experiment distributed to students, and a list of chemicals for the experiment (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

‡C.J.Y. is deceased.

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