

Elemental Analysis: Incorporating a Classic Analytical Technique into The Undergraduate Curricula

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Abstract: A laboratory exercise is described that involves the synthesis of acetylferrocene, analysis of its ¹H NMR spectra, and of its C and H composition by elemental analysis (EA). The use of both NMR and EA demonstrates to the students the value of using elemental analysis to evaluate the full purity of a sample in comparison to NMR that assesses purity by measuring the presence of one specific nucleus such as a proton. The use of EA also demonstrates the requirement to recrystallize the product of the synthesis in order to obtain a sufficiently pure product of acetylferrocene. This exercise gives students practice in synthetic techniques, instrumental analysis, the determination and interpretation of physical properties and in the critical evaluation of results obtained using different analytical techniques.

Introduction

In advanced undergraduate chemistry laboratory courses, student projects are often designed to provide a combination of experiences for the students. Such experiments may involve the synthesis of a compound and its characterization by analytical techniques; in many cases with an emphasis on modern methods and less frequently by classic techniques. The use of a comprehensive approach to the synthesis and analysis of a compound, and associated data interpretation offers numerous advantages in the learning process and simulates a realistic research methodology.

The synthesis of ferrocene and its derivatives, such as acetylferrocene are frequently included in advanced inorganic and organic laboratory courses [1, 2]. A useful supplement to student laboratory projects dealing with the synthesis of ferrocene and its derivatives is the quantitative determination of C and H by elemental analysis which can be used to evaluate the purity of the synthesized products. Elemental analysis, with automated combustion systems, provides a simple and rapid method for the determination of C and H, and also N and S in liquid or solid samples in a wide variety of matrices. The inclusion of the elemental analysis technique in a student project synthesizing an organometallic compound enhances the learning experience of the students, and allows the students to learn that a classic analytical technique is still necessary to prove purity.

CHNS elemental analyzers enable rapid determination of carbon, hydrogen, nitrogen and sulfur in organic matrices and other types of materials. They are capable of handling a wide variety of sample types, including solids, liquids, and viscous samples. These analyzers have been used in analytical laboratories for more than fifty years, and the method is

extensively used for a wide range of applications, including pharmaceuticals, chemicals, oil-related products, food and catalysts. In its simplest form, simultaneous CHNS analysis requires high temperature combustion in an oxygen-rich environment and is based on the classical Pregl-Dumas method. In the combustion process (at temperatures *ca.* 1000 °C), carbon is converted to carbon dioxide; hydrogen to water; nitrogen to nitrogen gas/oxides of nitrogen and sulfur to sulfur dioxide. If other elements such as chlorine are present, they are converted to combustion products, such as hydrogen chloride. A variety of absorbents are used to remove these additional combustion products as well as some of the principal elements such as sulfur if determination of these elements is not required.

In conventional combustion-based elemental analysis, the combustion products are swept out of the combustion chamber by an inert carrier gas such as helium and passed over heated (about 600 °C) high purity copper. The copper removes any residual oxygen not consumed in the initial combustion and converts oxides of nitrogen to nitrogen gas. The gases then pass through absorbent traps to remove undesired gaseous compounds or elements leaving only carbon dioxide, water, nitrogen and sulfur dioxide. Separation and quantitative measurement can be conducted using different procedures, such as (i) a gas chromatography (GC) separation followed by quantification using thermal conductivity detection, (ii) a partial separation by GC (“frontal chromatography”) followed by thermal conductivity detection (CHN but not S) and (iii) a series of separate infrared and thermal conductivity cells for detection of individual compounds. Quantification of the elements requires calibration for each element by using high purity standards compounds such as sulfanilic acid (41.6%, 4.1%, 8.1% and 18.5% for C, H, N and S, respectively).

Standard operating procedures for modern instruments are provided by their own manufacturers. They are easy to follow and easy to implement, allowing accurate results over the whole life of the instrument.

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Elemental analysis is a widely used analytical technique in many industrial and research applications, and is usually included for scientific publication of results in inorganic, organic and especially in organometallic synthesis. However, it is seldom features in undergraduate chemistry curricula. There is clearly a need for undergraduate chemistry students to understand this fundamental analytical technique and to learn how to interpret results. In order to provide students with an understanding of the principles and the use of elemental analysis, we have included a student project which uses direct determination of C and H by elemental analysis to assess the purity of the synthesized acetylferrocene. This demonstrates to the students the importance of elemental analysis to evaluate the purity of a substance.

Experimental Section

This experiment has been carried out at University of Almeria for several years in an Advanced Organic Chemistry Laboratory course. It is a course taken after Organic Chemistry I and II and consists of weekly three-hour laboratory classes. The preparation consists of the synthesis of acetylferrocene from ferrocene, the isolation of the product using column chromatography followed by the characterization and analysis of the product by ^1H NMR, thin layer chromatography (TLC) and CH elemental analysis [3]. Finally, a recrystallization protocol is performed in order to obtain analytically pure material which is followed by a second combustion analysis. The reaction chosen is the Friedel-Craft acylation of ferrocene. The catalyst used in the reaction is phosphoric acid, and the solvent used is acetic anhydride which when combined with the reagent allows for an electrophilic carbon-carbon aromatic substitution to occur. Procedures for the lab experiment were followed as described in reference 1 and are provided in the supporting material.

Schedule. The project is divided into four separate classes (Table 1, hereafter each class is referred to as a section). In the first section, the synthesis reaction is performed and TLC analysis is used to demonstrate that the reaction was not quantitative and that some ferrocene reagent was present with the desired product of acetylferrocene. In the second section, column chromatography is used to separate the reagent where every collected fraction is thoroughly analyzed by TLC and the desired product recrystallized in hexane. The third section has two parts; the first part is the preparation of samples for NMR and EA analysis (20–30 minutes). Every student prepares their own samples. In the second part groups of students work on the instruments for at least 1.5 hours to collect the data corresponding to their own samples, under the supervision of the corresponding technician. The theory behind and interpretation of the ^1H NMR spectroscopic and EA and combustion techniques are reserved for the fourth section of the project that includes some group discussion which assumes that the students have systematically worked through the interpretation. In the latter, the idea that ^1H NMR alone is not enough to support the assignment of a structure is an important one that upper level undergraduates students need to learn, and the need of other techniques such as ^{13}C NMR, HRMS or IR will be emphasized in this section.

Data Collection. Each student is given the reagents and glassware necessary to perform the synthesis [1], and all additional required material such as a 5 mm NMR tube and tin capsules. The students use an analytical balance of 1 microgram sensitivity, to prepare *ca.* 2 mg of the synthesized product before and after the recrystallization for elemental analysis.

The operation of each instrument (NMR and EA) is overseen by either the laboratory assistant or the specific NMR or EA technician. As each student runs their sample on the NMR instrument, the responsible laboratory assistant or technician indicates methods for acquiring the different spectra more quickly and provides the students with useful information of the theory and operation of each instrument.

Equipment. The NMR spectra are obtained with a Bruker Avance 300 MHz instrument controlled by XWINNMR 3.5 software. The EA data are obtained with an Elementar CHNS Micro Cube instrument controlled by Vario micro software. Analysis is performed once for every sample analyzed. The costs of the use of both the NMR spectrometer and the EA instrument can be found in reference 4.

Safety. Phosphoric acid [CAS # 7664-38-2] is corrosive and acetic anhydride [CAS # 108-24-7] is corrosive and a lachrymator, so it is necessary to avoid contact or undue exposure to vapors. Silica gel [CAS # 112926-00-8] may be irritating to skin, eyes and mucous membranes. Prolonged or repeated inhalation may cause damage to the respiratory system. Hexane [CAS # 110-54-3], diethyl ether [CAS # 60-29-7] and toluene [CAS # 108-88-3] are common organic solvents and should be handled accordingly; they should be kept away from flames, not inhaled, and disposed of in an organic waste container. The safety issues directly associated with the student's use of the EA relate to avoiding contact with hot inner surfaces; students will be informed of the necessity to keep the instrument completely closed to avoid being burnt. The technician responsible for the EA will explain to the students the safety issues associated with the management of the EA – the need for heat protection gloves when handling the oven and the combustion and reduction tubes when hot, and the need for gloves and a fume hood when handling desiccant material. The technician will explain that all manipulation of the components of the EA, when cold, requires the use of nitrile gloves to avoid skin contact. Saturated sodium bicarbonate [CAS # 144-55-8] solution may cause mild irritation to skin. Eye protection must be worn while performing this experiment. Gloves are suggested. Reagents should be dispensed in a hood to avoid inhalation of vapors.

Results and Discussion

Acetylferrocene is synthesized under mild conditions (see supporting information) with a modification of the procedure reported by Bozak [5] following reference 1. This encourages students to develop multi-tasking skills in the laboratory. TLC analysis qualitatively establishes that both the reagent ferrocene and an additional product, which is assumed to be acetylferrocene, are both present in the reaction crude.

The ^1H NMR spectrum of ferrocene consists of a single resonance peak at about δ 4.1 ppm. Although this resonance is 3 ppm upfield from a typical proton resonance in benzene, this difference is not the result in any decrease in aromaticity, but rather is explained by the fact that the higher electron density of the anionic rings result in greater shielding of the ring protons. The single resonance illustrates that all of the ring protons are in an equivalent chemical environment. The ^1H NMR spectrum of acetylferrocene show a singlet at δ 2.4 ppm with an integration of three protons from the acetyl group. Two pseudotriplets each of them with an integral of two protons were found at δ 4.5 and 4.8 ppm. The α -protons are more down field shifted than the β -protons due to the effect of the carbonyl group. In addition, a singlet with integration of five was found at δ 4.2 ppm due to the protons of the non-acylated ring. After analyzing the ^1H NMR results, the students make an assessment of the purity of acetylferrocene in the product. Their evaluation is subsequently confirmed or not by the use of EA.

Samples of the student's products are dried in a forced air oven at 40 °C for 30 minutes and then cooled in a desiccator. Each student weight out 2 mg of the sample into a tin capsule using a micro balance (accurate to 0.001 mg), and closes the capsule to form a tight ball. The students enter the details of their samples into the appropriate table in the Vario micro software and then introduce their capsules into the carousel of

Table 1. Distribution of the activities in each section and the sequence of the activities

No. of section	Duration (h)	Activities
Section 1	2h	Synthesis and TLC analysis
Section 2	2h	Column chromatography, TLC analysis and recrystallization
Section 3a	0.5h	Sample preparation for ^1H NMR and EA
Section 3b	1.5h	Experience with ^1H NMR and EA instruments
Section 4	1h	Theory and data interpretation

Table 2. Results obtained from the elemental analysis of acetylferrocene isolated from column chromatography. The theoretical value of acetylferrocene is 63.2% and 5.3% in carbon and hydrogen, respectively

Column-isolated			Recrystallized		
Code	C [%]	H [%]	Code	C [%]	H [%]
0-13-1-5b	66.0	5.7	0-13-1-5c	63.1	5.3
0-13-2-5b	75.6	6.4	0-13-2-5c	63.0	5.2
0-13-3-5b	61.7	5.4	0-13-3-5c	63.1	5.2
0-13-4-5b	66.1	5.6	0-13-4-5c	63.0	5.2
0-13-5-5b	65.7	5.8	0-13-5-5c	63.1	5.2
0-13-6-5b	61.4	5.4	0-13-6-5c	63.1	5.3
0-13-7-5b	66.1	5.6	0-13-7-5c	63.1	5.3

the EA. All of these activities are done under the supervision of the EA technician. The technician explains to the students, the functioning and operation of the instrument, and, in the context of the analysis being undertaken, the use of blank values and standards and the calibration procedure of the instrument. Each student sees part of the initial procedure of running blanks and standards and sees the analysis in real time of their own samples (see instructor notes in the supporting material). Students see the formation of the peaks pertaining to C and H of their samples, and observe the table with the complete set of results for the analytical run, on the instruments computer at the end of the analytical run.

The results of the C and H contents of eight student's samples for the initial attempt at synthesis of acetylferrocene are presented in Table 2. Also presented in Table 2 are theoretical values for acetylferrocene. In order to achieve analytical purity, the students take some of their own column-isolated acetyl ferrocene and recrystallized it in the minimum amount of hexane (see supporting information). Later on the students employ the crystals for EA, so then each student would have one NMR and two EA results and get to see the results of their own recrystallization skills. Table 2 includes the excellent empirical values afforded by all the students after the recrystallization process.

The results of the analysis of the products show notable differences between the composition of the non-recrystallized samples and that of the theoretical or recrystallized ones, as well as appreciable variation between the student's column-isolated samples.

These results demonstrate variable amounts of impurities in the student's non-recrystallized samples and that none of the samples were completely pure. Possible sources of the impurities present are residues of solvents, phthalate-type impurities and reagents. The results of the student's EA clearly demonstrate the requirement for recrystallization of the product of the syntheses and the importance of EA on evidencing it.

The students are taught to consider all of the data from both the NMR and EA analyses to arrive at the conclusion: "*The product is not pure and a recrystallization is needed*". In doing so, they learn that while NMR analysis characterizes the structure of a species it sometimes does not inform of purity.

This is a consequence of NMR observing only a selected nucleus, in this case a proton; and therefore any non-protonated impurity will not be observed.

Another aspect is the sensitivity associated to NMR. Organic impurities that only contribute to a couple percent of a mixture would not easily be detected by NMR but would be enough to make an elemental analysis fail. On the other way around, it might also be noted that an impurity that is an isomer of the intended product would not be detected by elemental analysis, but might be readily detected by NMR.

Each student writes a lab report that includes: (a) copies of the following data that relate to their own samples of all NMR spectra - the complete NMR report with tables that list the NMR peaks, identification of peaks, and the print-out of the EA results, and (b) a detailed explanation of the reasoning used to evaluate the purity of the final product. If there are any ambiguities in the proposed structure, completion of the reaction, or purity, these should also be included in the lab report. The report reflects the student's comprehension of the material covered in the last section and reinforces the advantages and limitations of each type of analysis.

Conclusions

The synthesis of acetylferrocene, a standard laboratory exercise in inorganic and organic laboratory courses, can easily be extended to include EA measurements. The results of elemental analysis of the student's synthesis products are inconsistent with the theoretical composition illustrating to students the requirement to recrystallize the product in order to obtain pure samples of acetylferrocene. By conducting this project, students become aware of the method of elemental analysis, which is a method that is commonly not considered in current undergraduate chemistry curricula. In addition, the students acquire hands-on experience in the use of an NMR spectrometer and an elemental analyzer. The data received from both NMR and EA enables students to evaluate the purity of a given substance, encourages them to explore their chemical understanding and enables them to acquire knowledge of additional techniques to enhance their formation as future, well-trained chemists.

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Supporting Information. Instructions for students and instructors, experimental procedures, hazards information, and ^1H NMR spectra for both ferrocene and acetylferrocene.

References and Notes

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2. (a) Davis, J.; Vaughan, D. H.; Marco, C. An Enhanced Chromatographic Technique for the Preparative Scale Purification of Acetyl Ferrocene. *J. Chem. Educ.*, **1995**, *72*, 266–267. (b) Newirth, T. L.; Srouji, N. Acetylation of Ferrocene: A Study of the Friedel-Crafts Acylation Mechanism as Measured by HPLC Using an Internal Standard. *J. Chem. Educ.*, **1995**, *72*, 454–456. (c) Hwa, R.; Weizman, H. Revisiting the Separation of Ferrocene and Acetylferrocene by Adsorption Chromatography: Adding a Third Component. *J. Chem. Educ.*, **2007**, *84*, 1497–1498. (d) Birdwhistell, K. R.; Nguyen, A.; Ramos, E. J.; Kobelja, R. Acylation of Ferrocene: A Greener Approach. *J. Chem. Educ.* **2008**, *85*, 261–262.
3. Additional characterization techniques such as ^{13}C NMR, HRMS and IR can also be applied to both the crude and pure products. In the laboratory report the students could discuss the limitations of each analytical technique, i.e. HRMS can tell you that a compound with the desired molecular formula is present, but it does not attest to purity. In the same line, IR would confirm the presence of the carbonyl group in the product, but again does not provide if it is pure or not.
4. For the use of the 300 MHz Bruker Avance NMR spectrometer: <http://cms.ual.es/UAL/universidad/serviciosgenerales/stecnicos/Pagina/PAGINA35841> (accessed July 2015). For the use of the Vario Micro Cube EA instrument: <http://cms.ual.es/UAL/universidad/serviciosgenerales/stecnicos/Pagina/SCI2050> (accessed July 2015).
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