



DEVELOPMENT OF DECISION-MAKING COMPETENCE IN COURSES OF SPECTROMETRIC ANALYSIS OF ORGANIC COMPOUNDS

Abstract

The aim of the present study was to develop a level of competence that is not normally found in graduate or post-graduate chemistry courses, through a didactic intervention in the discipline of spectrometric analysis of organic compounds. The methodology of the work consisted in the analysis of a sample of a compound unknown individually by each student. Whereas organic compounds spectroscopy is most often learned and practiced, this intervention allows students to become comfortable with ^1H and ^{13}C NMR, and IR spectroscopy, in addition to mass spectrometry. Throughout the didactic intervention the students, through a thematic seminary, presented the results of the analysis of the spectra from the different techniques used. With the skills acquired from completing this laboratory work, the students become well-prepared to perform spectroscopic analyzes in subsequent experiments encountered in the organic chemistry laboratory.

Keywords: Structural elucidation, Decision-making, Problem-based learning.

DESARROLLO DE COMPETENCIAS DE TOMA DE DECISIONES EN CURSOS DE ANÁLISIS ESPECTROMÉTRICO DE COMPUESTOS ORGÁNICOS

Resumen

El objetivo del presente estudio fue desarrollar un nivel de competencia que no se encuentra normalmente en los cursos de química de posgrado o postgrado, a través de una intervención didáctica en la disciplina de análisis espectrométrico de compuestos orgánicos. La metodología del trabajo consistió en el análisis de muestra de compuesto desconocido individualmente por cada estudiante. Mientras que la espectroscopía de compuestos orgánicos es más a menudo aprendida y practicada, la intervención permite que los estudiantes se sientan cómodos con la RMN ^1H y ^{13}C , y la espectroscopía IR, además de la espectrometría de masas. A lo largo de la intervención didáctica los estudiantes a través del seminario temático presentaron los resultados del análisis de los espectros de las diferentes técnicas utilizadas. Con las habilidades adquiridas de completar este laboratorio, están bien preparados para realizar análisis espectroscópicos en experimentos posteriores encontrados en el laboratorio de química orgánica.

Palabras claves: Elucidación estructural, Toma de decisiones, Aprendizaje basado en problemas.

Autores: Ricardo Oliveira Silva,^{A,*} Juliano Carlo Rufino Freitas,^{A, B, C*} Marcelo Felipe Rodrigues Silva,^A Patrícia Cristina Bezerra Silva,^A Etelino José Monteiro Vera Cruz Feijó Melo^A and João Rufino De Freitas Filho,^{C*}

^A Departamento de Química, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, 1235, 50670-901, Recife, PE, Brazil.

^B Centro de Educação e Saúde, Universidade Federal de Campina Grande, Olho D'água da Bica, s/n, 58175-000, Cuité, PB, Brazil.

^C Departamento de Química, Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros, Dois Irmãos, s/n, 52171-900, Recife, PE, Brasil.

* e-mails: juliano.carlo@ufcg.edu.br and ros@ufpe.br

DEVELOPMENT OF DECISION-MAKING COMPETENCE IN COURSES OF SPECTROMETRIC ANALYSIS OF ORGANIC COMPOUNDS

Introduction

Spectrometric techniques such as Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) are the principal tools used in the elucidation and identification of organic compounds (Silva, et al. 2009 and Souza, Ribeiro, 2007). The introduction to this content, in Chemistry courses, generally involves the instrumentation required to obtain spectra, as well as the theoretical framework involved in the technique. This is then followed by more specific information about the characterization of a functional group, such as the wave number of absorption (IR), the proposal of a fragmentation mechanism (MS), or a change in the resonance frequency, depending on the chemical environment (NMR). This strategy is also adopted by the textbooks that are generally used in these courses (Breitmaier, 2002; Silverstein, Webster, 2000 and Pavia et al., 2010). Souza and Ribeiro (2007) built a "general scheme for elucidation the structure of organic compounds using spectroscopy and spectrometry methods". In their paper, the authors described a systematic method to help students in the solving of a problem (spectral interpretation /structural elucidation). But, the spectra used were selected by teacher. Others authors (Lopes, Fascio, 2004 and Leal, et al. 2007) also suggested strategies to facilitate the teaching-learning process, but always focused on the spectral interpretation the students were never invited to choose the spectroscopic or spectrometric method.

In fact, it is very common for professors of these curriculum components to present a set of spectra (IR, MS and NMR) from a particular substance and ask students to determine the structural formula of the substance, based on the data given. This strategy is common in the traditional teaching approach, and it is also present in more sophisticated pedagogies. For example, Lucas and Rowley (2011) built an Enquiry-Based Learning (EBL) strategy to teach spectroscopy. They created different scenarios for developing the strategy, but the spectra used to solve the problem, selected by the teacher, were available to students. These strategies contribute significantly to the formation of good spectrum readers (interpreters). However, when the selection of which spectrometric techniques are required to elucidate a specific structure is analyzed, these strategies contribute very little. Students are never faced with the situation of selecting which spectra to use in the structural elucidation of a particular substance, and there is little material about the strategy and methodology that should be used to elucidate a structure (Breitmaier, 2002).

Spectroscopy however, is an integral component of undergraduate chemistry education (Zubrick, 2012). In lectures, students are commonly taught the theory behind various spectroscopic methods and how to analyze data. This provides students with an important opportunity to hone their problem-solving skills. On the other hand, the transition from this "on-paper" understanding of spectroscopy to performing spectroscopic analysis in the laboratory is daunting for most students.



Although examples of an introduction to a particular spectroscopic technique have been reported, a laboratory concept that introduces the combination of sample preparation and multiple spectroscopic techniques at the beginning of an organic chemistry laboratory course remains underdeveloped (Liotta; James-Pederson, 2008; Chamberlin, 2013 and Shine; Glagovich, 2005).

Here, it is necessary to distinguish and clearly relate competence, skills and knowledge. Competence and knowledge cannot be dissociated and are also not antagonistic. Knowledge of the various spectrometric techniques and how to interpret the information contained in the spectra is a basic and fundamental part of chemistry education, particularly for organic chemistry. Focus, up to the present, has been on knowledge and skills. Mastering these skills and this knowledge does not guarantee that the trainee professional will make the most appropriate decisions in due course. This is where competence comes into the equation. It is necessary to develop and build this competence. According to Perrenoud (1999), "competencies are acquisitions, built knowledge, and not virtual ties of the species". Decision-making competence is as important in professional life as it is in personal life, so it is necessary to provide opportunity to develop this competence (Bruin; Missier; Levin, 2012).

From the educational point of view, in this didactic intervention the student is responsible for the construction of knowledge, while the role of the teacher is as mediator in this process. This means that the teacher's interventions need to be provocative, prompting students to reflect on the problem presented.

Therefore, the specific learning objectives for the students are as follows:

- 1) Use fundamental laboratory techniques, including pipetagem and sample preparation, for spectroscopic analysis;
- 2) Understand theory and techniques of spectroscopic practice to elucidate organic compounds;
- 3) Interpret NMR, IV and GC-MS data from unidentified organic compounds;
- 4) Prepare and present a seminar on the elucidation of the structure of an unknown compound using different spectrographic techniques;
- 5) Develop confidence in and collaboration with other students in order to carry out challenging experiments.

METHODOLOGICAL APPROACH

General aspects of didactic intervention

The intervention proposal was developed with four students from the Chemistry post-graduate program of the Department of Fundamental Chemistry in the Federal University of Pernambuco (DQF-UFPE), in the discipline of spectrometric analysis of organic compounds, whose the objective was to develop the decision-making capacity of the students, share individual progress and promote collective conceptual growth through debates.



The didactic interventions were carried out in three moments, namely:

- a) Surveys of students' previous conceptions about the main spectrometric techniques and preparation of samples for analysis followed by a dialogical exposition on the subject;
- b) Implementation of experimental protocol;
- c) Fair presentation of results in the form of seminars.

The first moment was developed in two stages (conception surveys - two hours and dialogue exhibition - twenty hours). For the development of this experiment (second moment), the choice of compounds used for analysis was critical. More specifically, we sought a compound that

1. Would provide interesting, yet not unreasonably complex ^1H and ^{13}C NMR spectra.
2. Had diagnostic IR stretching frequencies representative of the common functional group, and
3. Was sufficiently volatile to undergo GC-MS analysis.

The optimized experimental protocol was implemented by the first in the discipline of organic compound spectrometry requires four hours to finish and can be performed over a single laboratory period.

Finally, in the third moment the students presented seminars with final results of their spectroscopic analysis of the compounds. This was carried out in two-hour classes.

Experimental

Material

The structures of the samples are described in Figure 1. The electro-thermal analogical equipment model Mel-Temp was used to determine the fusion point. IR spectra were obtained on KBr tablets (Aldrich), using a Bruker FT-IR IFS-66 spectrometer. A chromatograph, coupled to a Shimadzu GCMS-QP5050A mass spectrometer was used. The spectrometer was equipped with a capillary column DB-5MS, 30 m x 0.32 mm x 0.32 μm , with helium as the carrier gas and a flow of 1.0 mL min^{-1} . The split type injector was maintained at 250°C. The initial temperature of the oven was 60°C (0 min), with a heating rate of 10°C. min^{-1} up to 275°C (10 min). The high-resolution MS was obtained with a Shimadzu LCMS-IT-TOF spectrometer. The NMR 1D and 2D spectra were obtained using CDCl_3 , $\text{DMSO-}d_6$ and D_2O , with a Varian VNMRS400 spectrometer, equipped with a 5 mm-diameter PFG probe. All radio frequency pulse sequences used were provided by the manufacturer. All spectra were processed with the default parameters of the supplier.

Experimental Procedure

Students worked individually to complete this laboratory experiment. Students added five drops of a different unknown samples (sample A - student 1, sample B - student 2, sample C - student 3 and sample D - student 4) to a test tube containing 1.5 mL of

CDCl_3 . A portion was then poured into an NMR tube. Students labelled their samples, and submitted them to the teacher. The remaining solution of unknown sample in CDCl_3 could be used for IR spectroscopic analysis. One drop of the sample was loaded into the IR spectrometer equipped with ATR fixation. After evaporation of the CDCl_3 , an IR spectrum was obtained. To prepare samples for GC-MS, one drop of each sample was added to a standard GC-MS flask. The sample was then diluted with methanol (1 mL) and submitted for analysis.

Harzards

Deuterated chloroform (CDCl_3) is a cancer suspect and mutagen agent. Methanol is flammable and toxic by inhalation, contact with skin, or ingestion.

RESULTS AND DISCUSSION

The didactic intervention presented here is a typical Problem-based learning approach. The curriculum component was built around learning to choose appropriate MS, IR and NMR methods. Since the students had already encountered these techniques during their graduate courses, this phase was characterized by reminding them of what they had already studied exercising decision-making in choosing the most appropriate method.

The first moment that dealt with surveys of students' previous knowledge, we posed the following questions to the students: i) Do you know the different techniques for elucidating organic structures? ii) Do you understand the strengths and limitations of each of the analytical techniques you have used? iii) Did you need to learn other analytical techniques, and how did they help? According to the students' responses, the techniques of infrared, nuclear magnetic resonance, ultraviolet and mass spectrometry were known. But they could not provide details about the same. The students were shown the limitations of routine undergraduate characterization experiments, such as techniques using infrared spectroscopy, and some of the ^1D - and ^2D -NMR techniques.

In the second stage we discussed a number of relevant topics, such infrared spectroscopy, nuclear magnetic resonance (e.g., HMBC, APT), through dialogic expositions. In addition to the critical discussion of electromagnetic radiation, students were taught about the entire spectrum of electromagnetic radiation. They discussed nuclear magnetic resonance and infrared spectroscopy from a theoretical and practical standpoint. A discussion of proper sample preparation methods could not be overlooked, as these were new to most students. Similarly, the theory and practice of mass spectrometry was discussed, including explanations of commonly used instruments and accessories (e.g., GC-MS).

In the second moment, a simple experimental protocol intended to help students acclimate to undergraduate organic chemistry instructional laboratories was developed. The protocol had students analyze of unknown sample using ^1H NMR, ^{13}C NMR, and IR spectroscopy. In addition, GC-MS analyses were performed. In addition to using the IR spectrometer, students had the opportunity to see the NMR and GC-MS instruments in action (data collection for NMR and GC-MS spectra was performed by teacher).

In the third moment, the students were requested to prepare a two-hour seminar on the different techniques and their applications. During and after the presentation of these seminars, discussions were held to explore the potential of each technique.



Due to the importance of structural elucidation and the value of the information that can be obtained, NMR was discussed in three seminars. Since that the subject was not exhausted in these discussions, it was further discussed in other meetings during the course.

After this first phase, each student selected a sample identified only with the letters A, B, C and D, with its respective elementary composition (Table 1).

Physical state	Elemental composition (%)					
	C	N	O	H	S	Cl
Liquid	60.6	14.1	16.2	9.1	-	-
Solid	41.4	32.2	18.4	8.0	-	-
Solid	66.9	15.6	11.9	5.6	-	-
Solid	60.1	13.1	-	5.6	10.0	11.1

Table 1. Elemental composition and physical state of the substances used

Characterization and Elucidation of Substances

Each student used a different strategy to elucidate the structure, based on the initial information. Generally, the work was started by determining the empirical formula from the elemental composition, and by investigating the solubility of the compounds in different solvents. Each of these strategies is presented below, along with the route adopted by the students to characterize and elucidate the structure of each substance. This section confirmed the viability of the application of this strategy and the tested the cognitive development of the students with respect to making the appropriate decision.

Determination of the structure of substance A

The elemental composition of substance A indicated a minimal formula equal to C_5H_9NO (99g. mol⁻¹). After the solubility test, the student reported that the substance was soluble in chloroform and in ethyl acetate. Since the substance was in a liquid state, the student decided to use GC-MS, with ethyl acetate as solvent. The mass spectrum obtained had the peaks m/z 99 (55%), 41 (100%), 44 (55%) and 43 (43%), suggesting that the molecular weight was equal to 99g. mol⁻¹. In other words, the minimal formula was equal to the molecular formula. Using the molecular formula, it was possible to calculate the hydrogen deficiency index (HDI) equal to 2.

After this initial analysis, the student began the investigation of the structural formula by obtaining ¹H NMR spectrum and performed Attached Proton Test (APT). APT provides information about the number of magnetically similar carbons, as well as their multiplicity.

¹H NMR spectrum exhibited four signals as shown in Table 2. According to the student's analysis, from coupling constants (Table 2) recorded to the signals at δ 3.28 and 2.26, he concluded that these signals were coupled to the signal at δ 1.92, whereas the signal at δ 2.73 was possibly from a methyl group connected to oxygen or to nitrogen

nuclei, based on the relative integration area and the chemical shift. The APT spectrum exhibited five signals, indicating that all the carbons of the structure are magnetically different: one carbonyl group at δ 174.8; three methylene groups at δ 49.2, 30.4 and 17.4; and one methyl group at δ 29.3. Based on these data, the student concluded that there must have been a methyl group connected to nitrogen because the oxygen atom of this structure was found in the carbonyl. The couplings recorded in the ^1H NMR spectrum also indicated that the two methylene groups (δ 3.28 and 2.26) were interconnected by another methylene group (δ 1.92). Only one carbon with sp^2 hybridization was found. Therefore, based on the HDI value, the student deducted that the structure of substance A must be a cyclic chain.

The student concluded that the structure that met all these requirements was *N*-methyl-2-pyrrollidone. To confirm this, the student requested correlated spectroscopy (COSY) and HMQC experiments. The COSY spectrum confirmed the suggested couplings and the HMQC spectrum confirmed the attributions displayed in Table 2.

C or H	δ_{H} / ppm (m, J, n ^o)	δ_{C} / ppm
CH_3	2.73 (s, 3H)	29.3
C=O	-	174.8
CH_2	3.28 (t, 7.1, 2H)	49.2
CH_2	1.92 (m, 2H)	17.4
CH_2	2.26 (t, 8.0, 2H)	30.4

Table 2. Assignments of NMR signals for substance A

It must be pointed out that a definitive attribution would demand a heteronuclear multiple-bond correlation (HMBC) experiment to be conclusive, indicating which of the methylene groups (δ 2.26 or 3.28) presents HMBC correlation with the carbonyl nuclei (δ 174.8).

Determination of the structure of substance B

The data from the elemental analysis demonstrated an empirical formula equal to $\text{C}_3\text{H}_7\text{N}_2\text{O}$ (87g. mol⁻¹). The solubility tests revealed that the compound was highly soluble in water, but practically insoluble in most of the common organic solvents. This indicated that the substance B was very polar, which increased the difficulty of obtaining a mass spectrum by GC-MS. Therefore, the student elected not to request MS at this time and instead began the NMR spectroscopy, using D_2O as solvent. The following NMR spectra were requested: ^1H , ^{13}C and HMQC.

The ^{13}C NMR spectrum indicated that there were six magnetically different carbons in the structure, being three methylene groups (δ 24.44; 31.57; and 40.95), one carbon with sp³ hybridization (δ 55.53) and two non-hydrogenated carbon (δ 156.75 and 183.14) groups. This suggested that the molecular formula of the substance was twice its empirical formula ($\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$). This formula indicated an HDI equal to 2. The signals at δ 156.75 and 183.14 could be attributed to the groups C=N and carbonyl, respectively. However, this needed to be investigated further, and for this purpose an IR spectrum was requested.



¹H NMR spectrum exhibited two sets of signals with integration areas maintaining a ratio of 3:4. The first set exhibited between δ 3.30 and 3.00, and the second set centred at δ 1.53. The multiplicity observed for these signals suggested a higher order system. It was not possible to determine the coupling constants and the spin system by visual analysis alone. It was suspected that the signals belonged to the same spin system. For this reason, the student requested a COSY spectrum. This spectrum confirmed this suspicion and indicated that the set of signals between δ 3.30 and 3.00 was composed of two multiplets at δ 3.18 and 3.12. These were coupled together whereas the centred multiplet at δ 3.12 also coupled with a centred multiplet at δ 1.53. Therefore, the CH groups were in the same spin system, with an absence of nitrogen and/or oxygen atoms between them. Nitrogen and oxygen must beat the extremities of the chain, suggesting the presence of functional groups of amine, amide, nitrile, carboxylic acid or alcohol. The student excluded the functions ketone and aldehyde based on the fact that there would have had to be at least two spin systems.

In terms of the molecular formula, we conceded that there are hydrogen atoms in O–H and/or N–H form, since three methylene groups and one acetylene group only provided seven hydrogen atoms in the molecular formula, whereas the proposed formula indicated 14 hydrogen atoms in the molecule. Therefore, there would have to be seven hydrogen atoms in O–H and/or N–H form. Since the ¹H NMR spectrum was recorded in D₂O, these signals would not be observed in this spectrum due to the fact that hydrogen atoms had been replaced by 2H atoms.

The IR spectrum exhibited a broad band of about 3347 cm⁻¹, which can be associated with hydroxyl groups and/or N–H, as well as bands at 1648 cm⁻¹ and 1520 cm⁻¹, which can be attributed to carboxyl and C=N groups.

The student searched for the molecular formula C₆H₁₄N₄O₂ in SciFinder, to compare the molecular structures suggested by the SciFinder with the spectral data attained up to that moment. SciFinder provided 236 responses, only one of which was compatible with the spectral data previously collected: arginine. The student compared the spectral data found in the literature for arginine with the data that had been previously collected and concluded that the sample was arginine. Since arginine has a chiral centre, no further research was carried out to investigate the enantiomeric purity of the sample. This was not mentioned by the student in the report, although it was discussed in the classroom.

Determination of the structure of substance C

The student began the investigation by determining the empirical formula, melting point and solubility of the compound. This was done in order to select which spectroscopic technique to use. The elemental analysis indicated an empirical formula of C₁₅H₁₅N₃O₂. The melting point of the sample ranged between 193–195°C and the compound was soluble in chloroform. Based on these results, the student requested MS (using gas chromatography), IR, ¹H NMR and APT spectra. Mass spectrum was inconclusive. IR spectrum showed a combination of axial deformation at 3433 cm⁻¹, attributed to OH, and axial deformation at 1726 cm⁻¹, associated with C=O, indicating that substance C possibly contained an acidic function in its structure. IR spectrum also showed axial deformation at 1604 cm⁻¹, which could refer to COO⁻ or the connection of the conjugated C–C system in the aromatic ring. However, the former hypothesis of the COO⁻ was discarded due to other angular deformations of the aromatic rings at 818, 763 and 687 cm⁻¹.



The APT spectrum exhibited twelve signals: five were non-hydrogenated carbons between δ_c 170 and 120; six CH groups between δ_c 135 and 110; and one signal at δ_c 40.3 which could be assigned to a methyl or CH group. The 1H NMR spectrum exhibited a singlet, with a relative integration area equal to six at δ 3.19; and a set of signals between δ_h 8.5 and 6.5, with a relative integration area equal to eight. Using the data collected up to that moment, it was possible to infer the following about the groups present in the substance C:

- There were two chemically and magnetically equivalent methyl groups (δ_h 3.19 and δ_c 40.3) connected to the same nitrogen atom.
- There were two aromatic rings, one of which was para-substituted, considering that there was an AA'BB' system with signals at δ_h 7.80 (2H) and 6.76 (2H); and there was a second ortho-substituted aromatic ring with a spin system with four signals – δ_h 8.36 (dd, J 6.0, 1.2, 1H), 8.00 (dd, J 6.0, 0.6, 1H), 7.62 (td, J 6.0, 1.2, 1H) and 7.51 (td, J 6.0, 0.6, 1H).

The student also considered that if the ring were meta-substituted, one of the signals would not exhibit couplings in the order of 6-7 Hz, which is perfectly understandable. Figure 1 displays each of the units that constitute substance C:

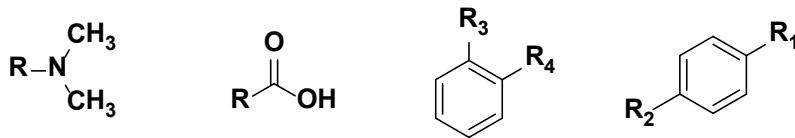


Figure 1. Groups present in substance C.

The empirical formula indicated 15 hydrogen atoms, although there were only 14 in 1H NMR spectrum, suggesting that one of the hydrogen atoms must have been represented as NH or OH. This confirmed the conclusion obtained from the IR spectrum related to the presence of carboxyl group. The APT spectrum indicated that there were five non-hydrogenated carbons. One of these was the carboxyl group (δ_c 167.4) whereas the other four (δ_c 154.1, 150.4, 142.1 and 125.2) were contained in the aromatic rings, with two substitutions in each ring. There were still two nitrogen atoms to be accounted for. Since the 15 hydrogen atoms were already allocated, it could be inferred that these nitrogen atoms were not connected to hydrogen atoms, but were probably interconnected by a double bond. There was no indication of the presence of nitrile or cyanide groups. Thus, by connecting the parts displayed in Figure 1 to the $-N=N-$ group, the result was two possible structures (Figure 2).

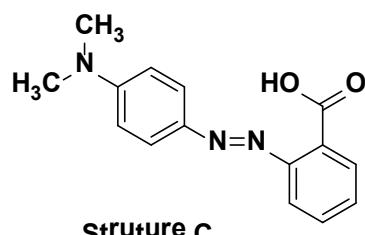
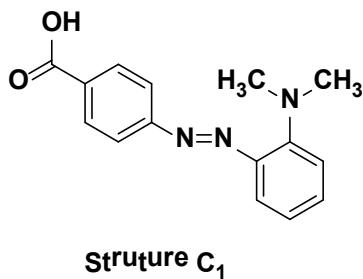


Figure 2. Possible structures of substance C.

In order to determine which of two structures, C1 and C2, he had, the student decided to request a HMBC spectrum (Figure 3), since the signal attributed to carboxyl (δ_c 167.4) needed to correlate with the hydrogen nuclei of the aromatic system.

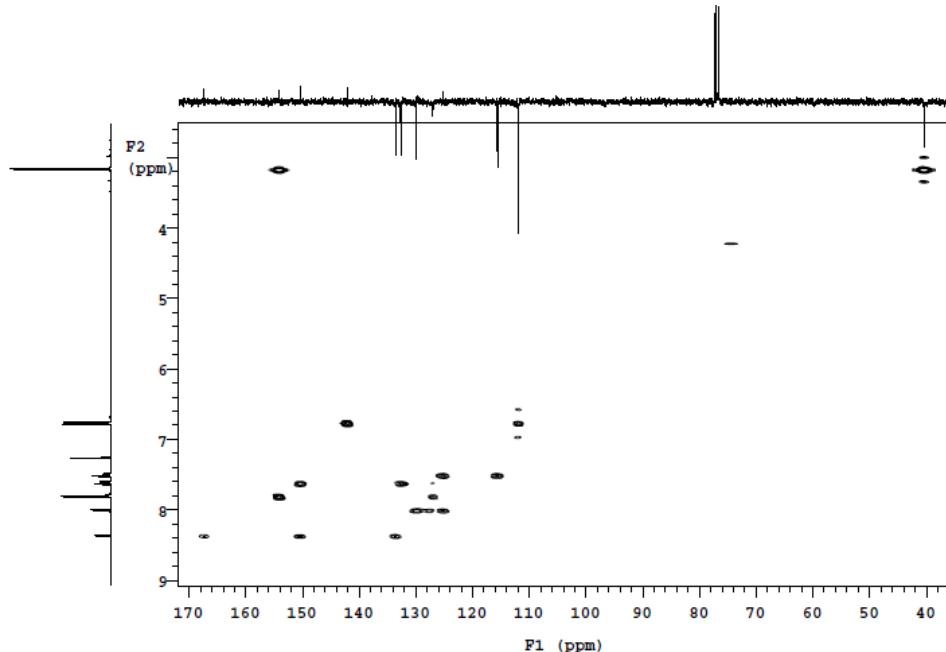


Figure 3. HMBC ^1H - ^{13}C spectrum (9 T, $\text{DMSO}-d_6$) of substance C.

HMBC spectrum shown correlation between the signals at δ_c 167.4 and δ_h 8.36 (ortho-substituted system), indicating that substance C had the C2 structure. It was also noticeable that the signal assigned to methyl groups (δ_h 3.19) correlated with a signal of δ_c 154.1, which in itself, exhibited a HMBC correlation with a signal of δ_h 7.80 (para-substituted system). This was a clear indication that the *N*-*N*-dimethylamine group was connected to the para-substituted system, and that substance C was methyl red.

Determination of the structure of substance D

The elemental analysis resulted in an empirical formula equal to $\text{C}_{16}\text{H}_{18}\text{N}_3\text{SCl}$, and a molecular weight of 319 g. mol⁻¹. Solubility test showed that the substance was soluble in water. Due to the molecular weight of the empirical formula and the solubility of the compound, the student requested high-resolution mass spectrometry by direct insertion. Mass spectrum exhibited a base peak with m/z equal to 284.1136 (M-35.7386).

This formula had HDI equal to 9. NMR spectra (^1H and ^{13}C), obtained in D_2O , exhibited 4 and 7 signals, respectively. Considering that there are 18 hydrogen and 16 carbon nuclei in the molecular formula, this reduced number of signals in the NMR spectra suggested that the structure was symmetric. Among the four signals in the ^1H NMR spectrum, three were as follows: δ 7.15 (d, J 9.2 Hz), 6.92 (δ , J 9.2 Hz) and 6.66, with a relative integration area of 1:1:1; whereas one was a singlet of δ 3.07, with a relative integration area of six. In comparison with the proposed molecular formula, the structure probably contained six hydrogen atoms in benzene rings and four methyl groups, which must have been chemically and magnetically similar and linked to nitrogen atoms due to the chemical shift



observed. Therefore, there must have been two dimethylamine groups in the structure. The rings must have been ortho-substituted and para-substituted based on the couplings found.

The ^{13}C NMR spectrum was consistent with the recorded statements, since it exhibited six signals between δ_c 160 and 100, and one signal of δ_c 40.5, which could be assigned to the methyl groups. Considering the HDI equal to 9, it was possible to imagine two isolated aromatic rings and one unsaturated ring, or three condensed rings. However, the latter would exceed the number of carbons contained in the molecular formula, considering the four methyl groups. Aside from chlorine, then, the following groups could be found in the structure of substance D (Figure 4).

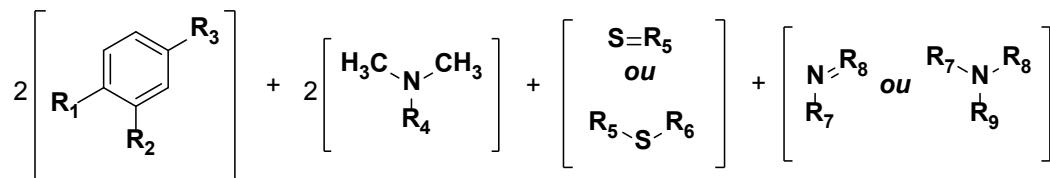
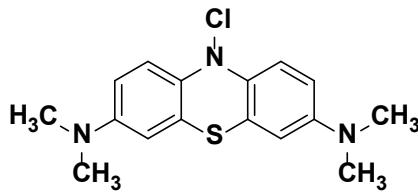
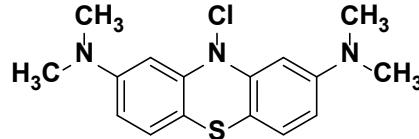


Figure 4. Groups present in substance D.

To meet the requirements of symmetry, very few structures could possibly be constructed with these groups. The student decided on two structures and was not able to determine which one was correct based on the analysis that had been performed up to that moment (Figure 5).



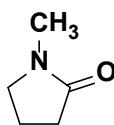
Structure D₁



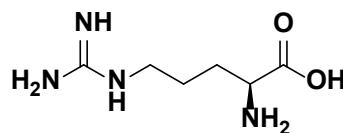
Structure D₂

Figure 5. Possible structures of substance D.

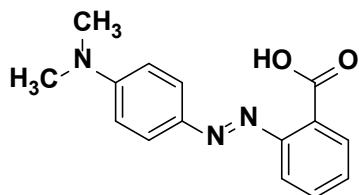
The student chose this route because the structures had an axis of symmetry (C2), which was consistent with the spectra obtained up to that moment. In principle, the two structures would provide very similar NMR spectra. The spectral option to solve this problem, as commented by the student in the report, would be to request a HMBC spectrum, however, the student opted to consult the literature and identified that structure D1 was methylene blue (acid-base indicator). Thus, the student added some drops of the solution used in the NMR experiments to solutions with different pH values. When the solution used was acid, there was a blue coloration whereas when the solution was alkaline, a pinkish coloration was observed. Based on this, the student concluded that substance D was methylene blue (Figure 6).



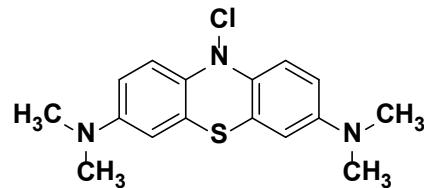
N-methyl-2-pyrrolidone
Substance A



Arginine
Substance B



Methyl red
Substance C



Methylene blue
Substance D

Figure 6 Structures of substances A, B, C and D.

After didactic intervention, students can were able to reconcile the structural composition to the physical properties and their implications. The students worked as individuals, guided by literature, andgot good results on a challenging problem. The students performed the sample preparation and analyzed the spectroscopic data to determine the structure of the unknown compound.

The didactic intervention discussed here has a simple, quick experimental procedure that did not overwhelm the students and calledfor the use of an unknown sample that required no preparation and could be easily obtained by teacher. Feedback received from the students and teachers was generally positive. They felt the exposure to NMR spectra and instrumentation was worth the class time spent. Depending on the skill level of the students, the experiment can be tailored to providedifferent levels of difficulty.

CONCLUSION

Students' comments on the didactic intervention evaluation were extremely positive. The recurring positive comments included:the experimental protocol and the seminar.

A simple experimental protocol intended to help students acclimate to undergraduate organic chemistry instructional laboratories was developed. The protocol consisted of analyses of unknown sample (sample A - student 1, sample B - student 2, sample C - student 3 and sample D - student 4) using ^1H NMR, ^{13}C NMR, IR spectroscopy and GC-MS.

By performing this laboratory work, studentswere exposed to a variety of common laboratory techniques and spectroscopic methods. Having completed this introductory experiment, students will have gained knowledge, competence, and confidence, without the added complexity of performing a chemical reaction.

Many factors seemed to contribute to the success of this didactic intervention, including: (1) The format of didactic intervention thatput responsibility for success on the students, while communicating high expectations; (2) facile access to technical support.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.



ACKNOWLEDGMENTS

The authors gratefully acknowledge CNPq (447361/2014-7), FACEPE-PRONEM (APQ-0476-1.06/14), CAPES and Analytical Center of the DQF-UFPE. Sidney Pratt, Canadian, MAT (The Johns Hopkins University), RSAdip - TESL (Cambridge University), for the revision of the text.

Fuente de financiación

CNPq (447361/2014-7), FACEPE-PRONEM (APQ-0476-1.06/14) and CAPES.

REFERENCES

Breitmaier, E. (2002). *Structure Elucidation by NMR in Organic Chemistry: A Practical Guide*, West Sussex: Wiley.

Bruin, W. B., Missier, F. & Levin, I. P. (2012). Individual Differences in Decision-making Competence. *Journal of Behavioral Decision Making*, 25(4), 329-330.

Chamberlain, P. H. (2013). Identification of an Alcohol with ^{13}C NMR Spectroscopy. *Journal of Chemical Education*, 90(10), 1365-1367.

Leal, K. Z., Yoneda, J. D., Seidl, P. R., Azeredo, R. B. V. & Kleinpeter, E. (2007). Camphor: a good model for illustrating NMR techniques. *Química Nova*, 30(8), 2053-2056.

Liotta, L. J. & James-Pederson, M. (2008). Identification of an Unknown Compound by Combined Use of IR, ^1H NMR, ^{13}C NMR, and Mass Spectrometry: A Real-Life Experience in Structure Determination. *Journal of Chemical Education*, 85(6), 832-833.

Lopes, W. A. & Fassio, M. (2004). Flow chart for infrared spectra interpretation of organic compounds. *Química Nova*, 27(4), 670-673.

Lucas, T. & Rowley, N. M. (2011). Enquiry-based learning: experiences of first year chemistry students learning spectroscopy. *Chemistry Education Research and Practice*, 12(4), 478-486.

Pavia, D. L., Lampman, G. M., Kriz, G. S. & Vyvyan, J. R. (2010). *Introdução à Espectroscopia*, CENGAGE Learning: São Paulo.

Perrenoud, P. (1999). *Construir as Competências desde a Escola*, Porto Alegre: ARTMED.

Silva, C. C., Silva, R. O., Navarro, D. M. A. F. & Navarro, M. (2009). Investigation of Unexpected Reaction Intermediates in the Alkaline Hydrolysis of Methyl 3,5-Dinitrobenzoate. *Journal of Chemical Education*, 86(4), 484-487.

Silverstein, R. M. & Webster, F. X. (2000). *Identificação Espectrométrica de Compostos Orgânicos*, 6th ed, LTC Editora, Rio de Janeiro.

Shine, T. D.; Glagovich, N. M. (2005). Organic Spectroscopy Laboratory: Utilizing IR and NMR in the Identification of an Unknown Substance. *Journal of Chemical Education*, 82(9), 1382-1384.

Souza, N. A. & Ribeiro, C. M. R. (2007). General scheme for elucidation the structure of organic compounds using spectroscopy and spectrometry methods. *Química Nova*, 30(4), 1206-1031.

Zubrick, J. W. (2012). *The Organic Chem. Lab Survival Manual: A Student's Guide to Techniques*, 9th ed.; John Wiley and Sons: Hoboken, NJ, Chapter 33.