

Application of ^1H -NMR for the Quantitative Analysis of Short Chain Fatty Acid Methyl Ester Mixtures: An Undergraduate Instrumental Analysis Experiment

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Abstract Nuclear magnetic resonance spectroscopy (NMR) is one of the most important instrumental techniques used to elucidate the molecular structure of organic compounds. However, there are not many good simple applications of using NMR for the quantitative assay of impure or mixtures of organic substances. The current experiment was developed as part of an ongoing effort to increase exposure to quantitative proton NMR methodology in the undergraduate chemistry laboratory curriculum. The objective of the experiment is to determine the percent composition of a two component mixture of short chain fatty acid methyl esters using chemical shift and integration values obtained from running solvent-less samples both with and without an internal reference standard. We report on the experimental methods and results obtained from examining the quantitative NMR properties of a series of mixtures of methyl acetate (M.A.) and methyl propionate (M.P.) solutions ranging from 100% MA to 100% MP. Also similar data was obtained for the MA - methyl butyrate (MB) pair. The results exhibit a linear relationship of the percent composition of the methyl acetate binary mixtures found by proton NMR with the theoretical (i.e. samples prepared based on measured weights) percent compositions. The experiment confirms the quantitative usage of ^1H NMR and serves as an educational tool for the undergraduate chemical laboratory.

Keywords: quantitative analysis, proton NMR, undergraduate laboratory experiment, hands-on learning, short chain fatty acid methyl ester mixtures

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1. Introduction

Although proton nuclear magnetic resonance (^1H NMR) spectroscopy is utilized for both qualitative and quantitative analyses of organic mixtures [1,2,3,4], there is a relatively limited number of quantitative experiments that can also be used as instructional exercises for undergraduate students [5,6,7,8,9]. The following ^1H NMR experiment was designed to support implementation of qualitative and quantitative NMR methodology in the undergraduate chemistry laboratory curriculum. The unique feature of NMR that allows its straightforward application to quantitative problems is that the signal's intensity is directly proportional to the specific number of protons producing that signal at the specific chemical shift. The current experiment determines the percent composition of a two-component mixture of short chain fatty acid methyl esters. Chemical shift and integration values were obtained by analyzing solvent-less organic samples with and without the use of an internal reference standard, followed by qualitative interpretation of peak assignments and splitting spin-spin coupling. Specifically,

the quantitative proton NMR properties of a series of methyl acetate (MA) and methyl propionate (MP) mixtures (ranging from 100% MA to 100% MP), as well as similar MA and methyl butyrate (MB) mixtures, were experimentally determined.

1.1. Learning Objectives

1. To learn how to properly prepare binary mixtures using volumetric and weight techniques
2. To understand the overall principles of proton NMR and the JEOL Delta software
3. To determine the chemical shift (δ), multiplicity and integration values of the assigned peaks
4. To establish correlation curves between the integrated NMR peak areas and the calculated weight % compositions for a binary mixture.

2. Materials and Methods

2.1. Overall Student Procedure

Each student will prepare approximately 20%, 40%, 60%, and 80% by mass of a mixture of either MA-MP or

MA-MB as assigned by the instructor. The student will establish a correlation curve of weight % composition of MA-MP or MA-MB versus weight % determined from NMR. The student will then analyze an unknown sample provided by the instructor and determine the % composition of MA present within the assigned unknown.

2.2. Experimental Materials and Chemicals

The short chain fatty acid methyl esters of methyl acetate ($\text{CH}_3\text{COOCH}_3$), methyl propionate ($\text{CH}_3\text{CH}_2\text{COOCH}_3$), and methyl butyrate ($\text{CH}_3\text{CH}_2\text{CH}_2\text{COOCH}_3$) were purchased from Sigma-Aldrich as reagent plus grade anhydrous liquids having greater than 99% purity. Tetramethylsilane (TMS), $\text{Si}(\text{CH}_3)_4$, was purchased from Sigma-Aldrich as reagent grade quality having a purity of greater than 99.9%. TMS was used as an internal NMR standard during the experiment. All reagents were used without further purification. The NMR sample tubes were Wilmad Pyrex glass 5mm x 7" thin wall tubes.

2.2.1. Proton NMR

The ^1H NMR profiles of the short-chain fatty acid methyl ester binary mixtures were obtained using a JEOL 400 MHz model ECS-400 NMR spectrometer. The JEOL Delta NMR control and process software version 5.0.2 (Windows) was used to analyze individual spectrum. Each sample was run in the absence of solvent as a single pulse, 1D proton NMR with a 0.25 Hz resolution and a relaxation time ranging from 8 and 10 seconds. Any vendors NMR hardware and software can be used to perform this experiment.

2.2.2. Experimental Procedure

Eleven 5.0 mL binary mixtures of methyl acetate (MA), methyl propionate (MP), and methyl butyrate (MB) were prepared by the students as shown in Table 1. Each reagent was subsequently added using a Gilson classic model P5000 pipette to a 7.5 ml empty glass vial containing a Teflon lined screw cap. Appropriate volumes of methyl propionate were added to the labeled 7.5 ml sample vials 1B thru 6B followed by the appropriate volume of MA. Similarly, appropriate volumes of methyl butyrate were added to samples vials 7B thru 11B followed by the appropriate volume of MA.

Table 1. Composition of the first set of methyl acetate (MA), methyl propionate (MP), and methyl butyrate (MB) binary mixtures

Sample Vial #	Volume (mL)			Experimental Weight Percent (%) Composition		
	MA	MP	MB	MA	MP	MB
1B	5.0	0	-	100.0	0	-
2B	4.0	1.0	-	81.5	18.5	-
3B	3.0	2.0	-	63.8	36.2	-
4B	2.0	3.0	-	43.8	56.2	-
5B	1.0	4.0	-	27.0	73.0	-
6B	0	5.0	0	0	100.0	0
7B	4.0	-	1.0	80.9	-	19.1
8B	3.0	-	2.0	61.7	-	38.3
9B	2.0	-	3.0	41.5	-	58.5
10B	1.0	-	4.0	22.4	-	77.6
11B	0	-	5.0	0	-	100.0

Following each chemical addition, the mass of each sample was determined using an analytical balance having

a precision of 10^{-3} g. The masses were used to establish the percent compositions of the binary mixtures.

The procedure was repeated by the instructor for a separate set of six samples to be used as student "unknowns" (Table 2). The masses of the short-chain fatty acid methyl esters from the binary mixtures were used to determine the percent composition of each sample tube.

A 1-ml aliquot of each mixture was transferred to the ^1H NMR tube using a Gilson classic P1000. 200 μL of TMS was added to a labeled NMR tube using a Gilson classic model P200 pipette.

Table 2. Second set of methyl acetate (MA), methyl propionate (MP), and methyl butyrate (MB) solutions to be used as student unknown samples

Sample #	Volume (mL)			Percent (w/w %) Composition		
	MA	MP	MB	MA	MP	MB
1C	3.75	1.25	-	72.8	27.2	-
2C	2.50	2.50	-	49.5	49.5	-
3C	1.25	3.75	-	23.8	76.2	-
4C	3.75	-	1.25	74.8	-	25.2
5C	2.50	-	2.50	50.0	-	50.0
6C	1.25	-	3.75	25.1	-	74.9

2.2.3. Hazards

Tetramethylsilane (CAS# 75-76-3), methyl acetate (CAS# 79-20-9), methyl propionate (CAS# 554-12-1), and methyl butyrate (CAS# 623-42-7) are all flammable liquids and harmful if swallowed or inhaled. Safety glasses are mandatory and the use of hoods is advisable. Students are required to wear protective gloves during the experiment. Waste solutions containing flammable liquids must be collected for waste disposal according to EPA and local guidelines. NMR magnets may interfere with electronic and metallic implants, so those students who have them should not come near the NMR magnet at any time.

2.2.4. Statistical Analysis

Each of the NMR samples prepared was analyzed multiple times. The data reported is an average of the normalized integrated peak areas obtained. The average percent relative standard deviation for the samples analyzed ranged from 0.2% - 0.8%.

3. Results and Discussion

Figure 1, Figure 2 & Figure 3 show the proton NMR spectrum, having TMS as the internal reference standard (IRS), of 100% methyl acetate, 100% methyl propionate, & 100% methyl butyrate respectively. The figures also include corresponding peak assignments. Summarized in Table 3 are the chemical shift with TMS as IRS, multiplicity and normalized integration values for each of the assigned peaks.

The methyl protons (A) on the carbon atom attached to the oxygen all are singlets and the chemical shift values are basically the same and have a normalized integration value of one. These protons are downfield (at higher frequency values) because the electron density of the environment in which these protons are located is less shielded from the applied magnetic field due to their proximity to oxygen. However, the methyl protons on the

parent chain of the esters (B on MA, C on MP, and D on MB) are shifting up-field, or lower frequencies as the molar mass of the fatty acid methyl ester increases. These protons are shielded and they sense a smaller effective magnetic field as the number of methylene groups is

added to the ester. Therefore these protons come into resonance at a lower frequency. Figure 4, Figure 5, and Figure 6 are the proton NMR spectrums, with TMS as the internal stand, of 1:4 MA/MP mixture, 4:1 MA/MP mixture and a 1:1 MA/MB mixture respectively.

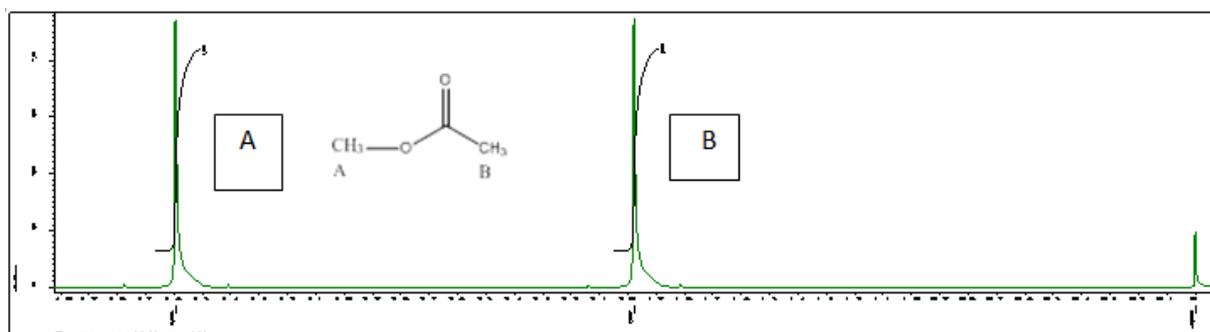


Figure 1. Proton NMR of 100% Methyl Acetate

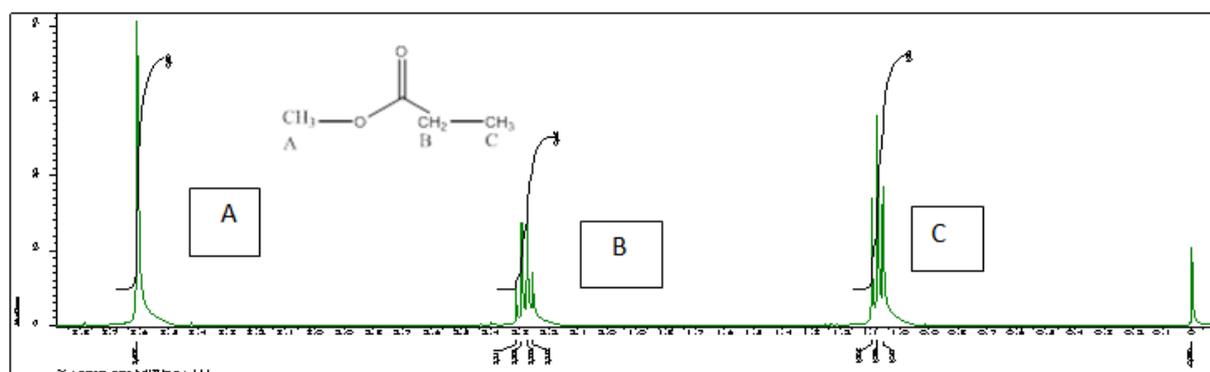


Figure 2. Proton NMR of 100% Methyl Propionate

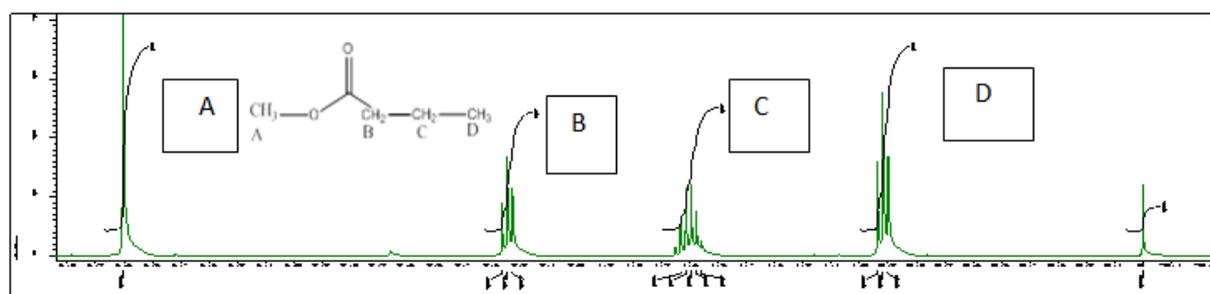


Figure 3. Proton NMR of 100% Methyl Butyrate

Table 3. Summarized in table below are the chemical shift, multiplicity and normalized integration values for each of the assigned peaks in MA, MP, MB

Methyl Acetate (MA)				Methyl Propionate (MP)				Methyl Butyrate (MB)			
Proton	Shift(δ) ppm	Multiplicity	Integration	Proton	Shift(δ) Ppm	Multiplicity	Integration	Proton	Shift(δ) Ppm	Multiplicity	Integration
A	3.596	singlet	1.00	A	3.607	singlet	1.00	A	3.592	singlet	1.00
B	1.979	singlet	1.00	B	2.273	quadruplet	0.65	B	2.255	triplet	0.64
				C	1.076	triplet	1.00	C	1.612	Multiplet	0.67
								D	.918	triplet	1.00

The % compositions were determined by the normalized integration areas of the methyl peaks, CH₃, for the short chain fatty acid moiety at 1.97 ppm for the

acetate (MA), 1.07 ppm for the propionate (MP) and .92 ppm for the butyrate (MB). The normalized integration of the appropriate peaks corresponds to the % composition of

the mixtures. The normalized integration of the appropriate peaks labelled MA, MP, MB in Figure 4, Figure 5, and Figure 6 correspond to the percent composition of the mixtures. Summarized in Figure 7 is the linear plot of the % composition of the MA/MP mixtures found by proton NMR vs the theoretical

(prepared) % compositions. Shown in Figure 8 is a similar plot for the MA/MB compositions. One can see that there is excellent agreement between the % compositions determined from NMR with the prepared weight % values. The correlation coefficient (R^2) in both cases were greater than .99.

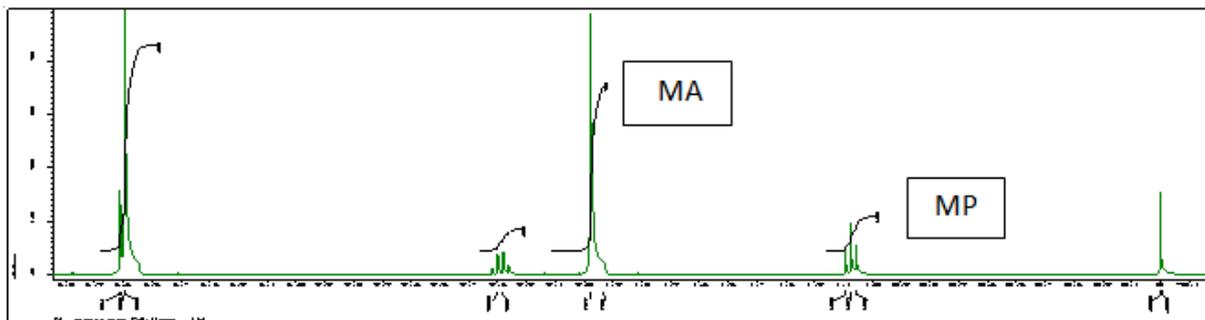


Figure 4. Proton Spectrum of Sample 2B. A mixture of 1 part MA and 4 parts MP

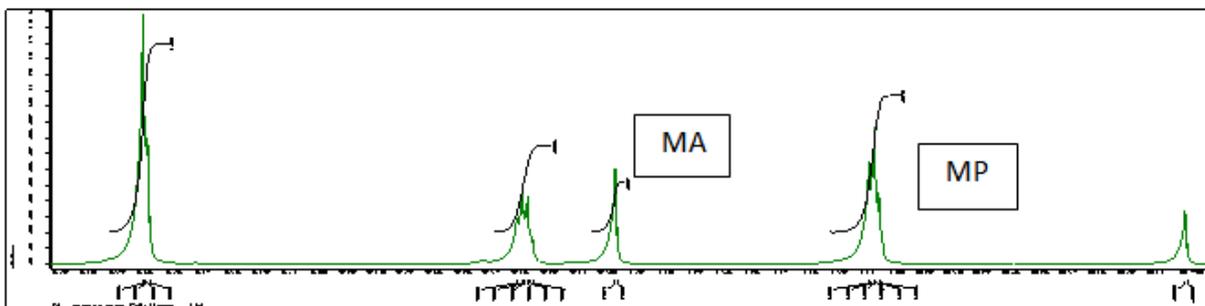


Figure 5. Proton Spectrum of Sample 5B. A mixture of 4 parts MA and 1 part MP

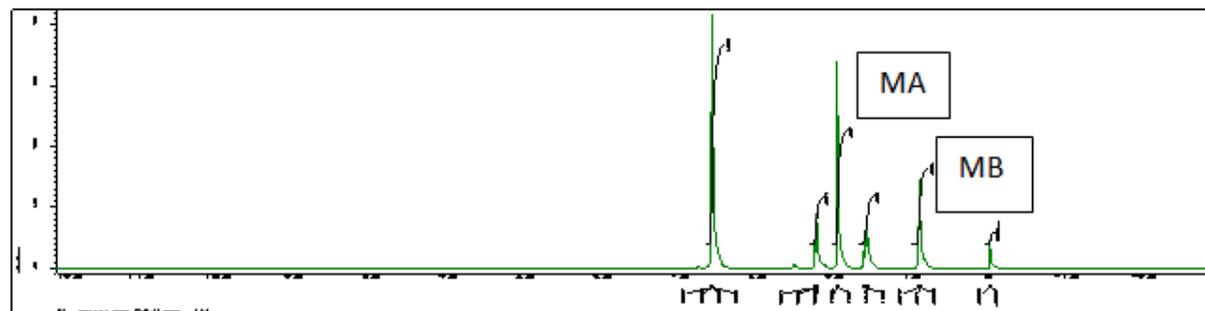


Figure 6. Proton Spectrum of Sample 5C. A mixture of equal parts of MA and MB

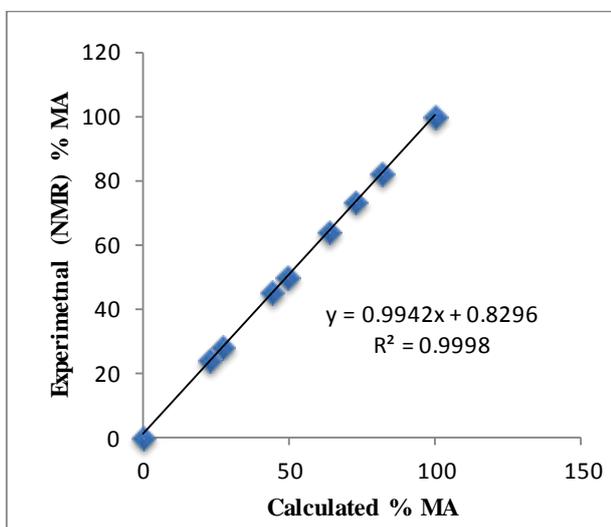


Figure 7. % Composition of M.A./M.P

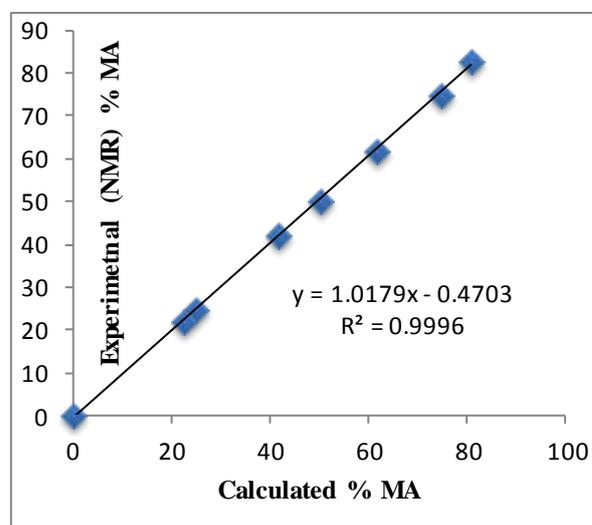


Figure 8. % Composition of M.A./M.B

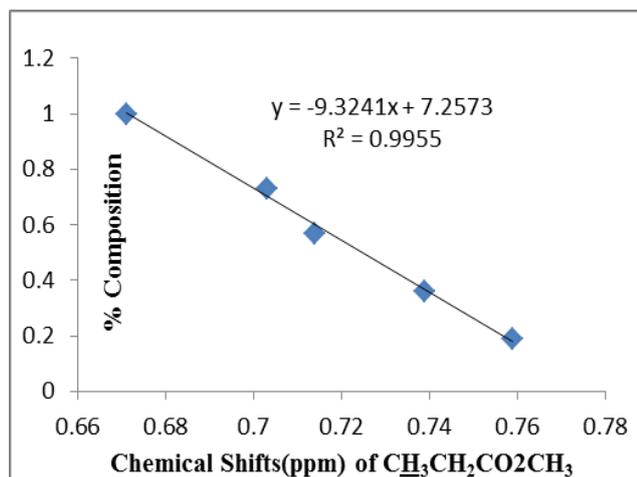


Figure 9. Plot of change in Chemical Shift of Methyl Proton on M.P. vs % Composition of M.P. with M.A. No TMS

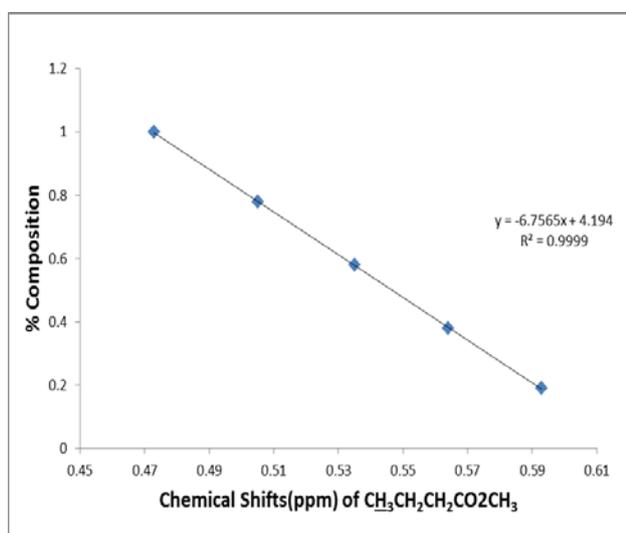


Figure 10. Plot of change in Chemical Shift of Methyl Proton on M.B. vs % Composition of M.B. with M.A. No TMS

We also ran the same samples in Table 1 without any internal standard (TMS). One finds that the terminal methyl protons on the acid side of MP and MB shift linearly up-field as the methyl protons get more shielded from increasing amounts of surrounding MP and MB, the amount of MA drops accordingly. Shown in Figure 9 is the linear plot of the change in chemical shift values of the methyl proton on MP (without TMS) vs composition. Shown in Figure 10 is the linear plot of the change in chemical shift values of the methyl proton on MB vs the composition. Both of these plots show the linear relationship in the up-field changes in chemical shift values that occur when no internal reference is considered.

These calibration curves can potentially be used to quantify components in a binary mixture or generate relative ratios of similarly structured short chain fatty acids.

4. Conclusions

The data has reaffirmed the quantitative uses of the NMR. A strong linear correlation between the actual and theoretical compositions of each mixture was observed as shown in the above graphs. Also, there is a direct linear correlation between the chemical shift values and proportional changes in the compositions of each mixture. Overall, this simple experiment confirms the quantitative usage of ^1H NMR and serves as an excellent tool for the undergraduate chemical laboratory.

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References

- [1] McGregor, M.A., Euler, W.B. *Undergraduate NMR Laboratory Experiments*. In *NMR Concepts*; Traficante, D.D. Ed.; NMR Concepts: Warwick, RI, 1995.
- [2] Woodworth J.K., Terrance J.C., and Hoffmann M.M., "Using Nuclear Magnetic Resonance Spectroscopy for Measuring Ternary Phase Diagrams", *J. Chem. Educ.*, 83(7), 1065-1066, July 2006.
- [3] Isaac-Lam M.F., "Analysis of Bromination of Ethylbenzene using a 45 MHz NMR Spectrometer: An Undergraduate Organic Chemistry Laboratory Experiment", *J. Chem. Educ.*, 91, 1264-1266, July 2014.
- [4] Gift A.D., Stewart S.M., and Bokashanga K.P., Experimental Determination of pK_a Values by use of NMR Chemical Shifts, Revisited", *J. Chem. Educ.* 89, 1458 – 1460, August 2012
- [5] Clarke, D., "Acetone and Ethyl Acetate in Commercial Nail Polish Removers: A Quantitative NMR Experiment using an Internal Standard", *J. Chem. Educ.*, 74(12), 1464-1465, December 1997.
- [6] Hoffmann M.M., Caccamis J.T., Heitz M.P., and Schlecht K.D., "Quantitative Analysis of Nail Polish Remover using Nuclear Magnetic Resonance Spectroscopy Revisited", *J. Chem. Educ.*, 85(10), 1421-1423, October 2008.
- [7] Phillips J.S., Leary J.J., "A Qualitative – Quantitative ^1H - NMR Experiment for the Instrumental Analysis Laboratory", *J. Chem. Educ.*, 63(6), 545-546, June 1986.
- [8] Bauner, B. and Pringle, D. "Proton NMR Analysis of Heat Exchange Fluids Containing Ethylene Glycol, Propylene Glycol, and Water: A Real-World Experiment for the Analytical Laboratory", *J. Chem. Educ.*, 91, 743-746, April 2014.
- [9] Podgorski, V.V., Milhalev, A.S., Kalabin, G.A., "Quantitative NMR Spectroscopy for the Quality control of Drugs and Pharmaceuticals", *Phar. Chem.J.*, 45(3), 194-197, June 2011.
- [10] Peterson, J., " ^1H NMR Analysis of Mixtures using Internal Standards", *J. Chem. Educ.*, 69, 843-845, October 1992.