

Application of Quantitative Proton Nuclear Magnetic Resonance Spectroscopy for the Compositional Analysis of Short Chain Fatty Acid Benzyl Ester Mixtures

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Abstract Nuclear Magnetic Resonance (NMR) has become one of the cornerstones of instrumental analysis work done in chemistry laboratories. NMR is most powerful when used quantitatively, the technique of using NMR to quantify the concentration of an analyte is referred to as qNMR and proton NMR, in particular, is extremely useful in this pursuit to quantify organic compounds. In order to increase exposure to the quantitative and qualitative aspects of NMR in the undergraduate chemistry laboratory, we have created a qNMR experiment to be used in analytical chemistry and instrumental analysis courses. The objective of the experiment was to determine the % composition of a two-component mixture of benzyl acetate (BA), benzyl propionate (BP), and benzyl butyrate (BB). We report on the methodologies used to determine % BA, BP, and BB. Mixtures ranged from 100% to 20%. The results show a strong linear relationship relating known weight %'s with qNMR weight %'s and serves as confirmation of the quantitative utility of proton NMR as well as an educational tool for the undergraduate chemical laboratory.

Keywords: quantitative analysis, Nuclear Magnetic Resonance, undergraduate laboratory experiment, hands-on learning, benzyl esters, benzyl ester mixtures

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1. Introduction

Proton Nuclear Magnetic Resonance Spectroscopy has been utilized for both qualitative and quantitative analyses of organic mixtures [1-5], however, there is a lack of reliable exercises using qNMR with the purpose of educational instruction [6-11]. In order to combat this, this experiment has been designed for simple implementation of the qNMR methodology into undergraduate chemistry laboratory curriculums. The quantitative utility of NMR comes from the fact that each peak's integration value is directly proportional to the number of protons producing that signal at that chemical shift value. This experiment determines the weight % composition of a two-component mixture of short-chain fatty acid benzyl esters. Chemical shift and integration values were obtained via running all samples neat with and without an internal reference standard. The peaks were assigned to specific protons found within the benzyl esters and a qualitative interpretation of the NMR spectra was done analyzing multiplicity, spin-spin coupling and integration values. Mixtures of BA, BB; BA, BP; and BB, BP were made ranging from 20 to 100%.

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 both qualitatively and quantitatively as well as the JEOL Delta software.
3. To determine the chemical shift (δ), multiplicity and integration values of the assigned peaks, relating them to the structure of the components of the binary mixtures.
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. Student Procedure

Each student will aliquot mixtures of approximately 20, 40, 60, and 80% by volume of a mixture of either BA-BP, BA-BB, or BB-BP. 7 mL sample vials will be weighed initially then following each addition of reagent the vial

will be weighed again to determine weight %. Students will then run proton NMR on their samples and develop a calibration curve of calculated weight % of the reagent of interest and the weight % determined via NMR. Following the development of the calibration curve, students will receive an unknown sample created by the instructor and using the curve they will determine the unknown weight %.

2.2. Experimental Materials

Benzyl acetate, propionate, and butyrate were purchased from Sigma-Aldrich as anhydrous liquids with greater than 99% purity. All reagents were used without purification. The NMR tubes used in this study were Wilmad Pyrex glass 5 mm x 7" thin wall tubes.

2.2.1. Proton NMR

The proton NMR spectra were obtained using a 400 MHz JEOL model ECS-400 NMR spectrometer. The JEOL Delta NMR control and process software version 5.0.2 (Windows) were 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 of NMR hardware and software can be used to perform this experiment.

2.2.2. Experimental Procedure

Fifteen 5.0 mL binary mixtures of benzyl acetate (BA), benzyl propionate (BP), and benzyl butyrate (BB) were prepared as shown in Table 1. Each reagent was added using a Gilson classic model P1000 pipette and 1 mL was added to NMR tubes for qNMR analysis. All 7 mL vials and NMR tubes were labeled with the appropriate volumetric ratio of reagents in the mixture. Following each chemical addition, the mass of each sample was recorded using an analytical balance having a precision of 0.1 mg. These masses were used to determine the precise weight percent composition of the mixtures. All mixtures were analyzed with and without an internal reference standard. The internal reference standard of choice for this experiment was tetramethylsilane (TMS) and was added to each sample in 0.2 mL quantities via a Gilson Classic model P200 pipette.

To create the unknown a 50-50 mixture of BA-BP, BA-BB, and BB-BP were created by the instructor as shown in Table 2.

2.2.3. Hazards

All of the benzyl esters used in this experiment are used consistently in commercial products such as cosmetics and perfumery products as such they do not pose any significant health risks, goggles are mandatory however in order to avoid exposure to the eyes and care should be made to avoid exposure to the skin. Tetramethylsilane, however, is flammable and is harmful if swallowed or inhaled, thus the use of a hood is advisable and safety glasses are mandatory. Waste solutions should be disposed of according to EPA and local guidelines. The NMR magnetic fields may interfere with metallic implants; students who have them should not be in the same room as the NMR at any time.

2.2.4. Statistical Analysis

Each of the binary mixtures was analyzed multiple times via NMR. The data reported is the average of the normalized peak areas with a standard deviation ranging from 0.22 to 0.95. All calibration curve predictions had a percent error of less than 2% relative to the gravimetric weight %.

Table 1. Summary of Aliquotted Binary Mixtures

Volume ratio	Experimental weight % Benzyl acetate
Benzyl acetate: benzyl propionate	Experimental weight % Benzyl acetate
0ml:5ml BA:BP	0
1ml:4ml BA:BP	23.779
2ml:3ml BA:BP	41.149
3ml:2ml BA:BP	61.019
4ml:1ml BA:BP	80.450
5ml:0ml BA:BP	100
Benzyl acetate: benzyl butyrate	Experimental weight % Benzyl acetate
0ml:5ml BA:BB	0
1ml:4ml BA:BB	20.582
2ml:3ml BA:BB	42.842
3ml:2ml BA:BB	61.674
4ml:1ml BA:BB	80.945
5ml:0ml BA:BB	100
Benzyl butyrate: benzyl propionate	Experimental weight % Benzyl butyrate
0ml:5ml BB:BP	0
1ml:4ml BB:BP	17.750
2ml:3ml BB:BP	38.863
3ml:2ml BB:BP	58.647
4ml:1ml BB:BP	79.579
5ml:0ml BB:BP	100

Table 2. Summary of Aliquotted Unknown Binary Mixtures

Volume Ratio	Experimental weight % Benzyl acetate
Benzyl acetate: benzyl propionate	Experimental weight % Benzyl acetate
1 ml:1 ml BA: BP	51.906
Benzyl acetate: benzyl butyrate	Experimental weight % Benzyl acetate
1 ml:1ml BA:BB	51.883
Benzyl propionate: benzyl butyrate	Experimental weight % Benzyl propionate
1 ml:1ml BP:BB	51.088

3. Results and Discussion

Figure 1 through Figure 3 depict the NMR spectra of 100% benzyl acetate, propionate, and butyrate respectively. Table 3 summarizes the chemical shifts, multiplicities, and normalized integration values for each peak in the neat samples. All reagents had a group of highly de-shielded protons near 7.0 ppm this is due to the benzyl ring being very electron withdrawing owing to its aromatic nature, the end result is the protons in the benzene ring are extremely de-shielded from the external magnetic field caused by the NMR, shifting them more downstream than one might expect. Each reagent also

had a peak around 5.0 ppm and this corresponds to the methylene group in between the benzene ring and the oxygen of the ester functional group. These protons are more de-shielded due to their proximity to the benzene ring, however, they are not as downstream as the benzyl protons due to them not being in full contact to the pi electron ring of the aromatic benzene ring. The main difference between BA, BP, and BB is the signals after the methylene protons adjacent to the benzene ring. In BA, there was only one signal after the methylene protons, this had a chemical shift value of around 1.8 ppm. The chemical shift value of these protons is less than that of the methylene and benzyl protons due to the fact that they are the farthest removed from the electron withdrawing effects of the aromatic benzene ring, resulting in a more shielded chemical environment. This peak was a singlet with a normalized integration value of 3 because the terminal methyl protons have no proton neighbors, thus there would be no spin-spin coupling resulting in a multiplicity higher than a singlet. In BP, there were two signals after the methylene protons adjacent to the benzene ring. These signals do exhibit multiplicities greater than 1 due to the fact that they do have neighbors. The signal that is farther downstream with a chemical shift

value of 2.1 ppm represents the protons that are closer to the carbonyl carbon of the ester group and due to the fact that those protons can spin-spin couple with the protons on the terminal methyl group of BP, the signal is a quartet as per the $n+1$ rule of 1D proton NMR. The terminal methyl protons are represented by the signal at 0.96 ppm with a multiplicity of a triplet due to the two neighboring protons of the previous methylene carbon adjacent to the carbonyl carbon. This signal is closer to 0.0 ppm due to the fact that it is farther from the electron withdrawing benzene ring as previously stated. In BB, there are three signals after the methylene protons adjacent to the benzyl ring. All of these signals exhibit a multiplicity greater than 1 due to neighboring protons. The signal that has a chemical shift value of 2.1 ppm represents the protons that are closer to the carbonyl carbon of the ester group and due to the fact that those protons can spin-spin couple with the protons of the adjacent methylene carbon, it is expressed as a triplet. The signal with a chemical shift value of 1.6 represents the protons in between the methylene carbon adjacent to the carbonyl carbon and the terminal methyl group and is a multiplet due to the 5 neighboring protons. The signal with a chemical shift value of 0.85 ppm and is a triplet due to 2 neighboring protons.

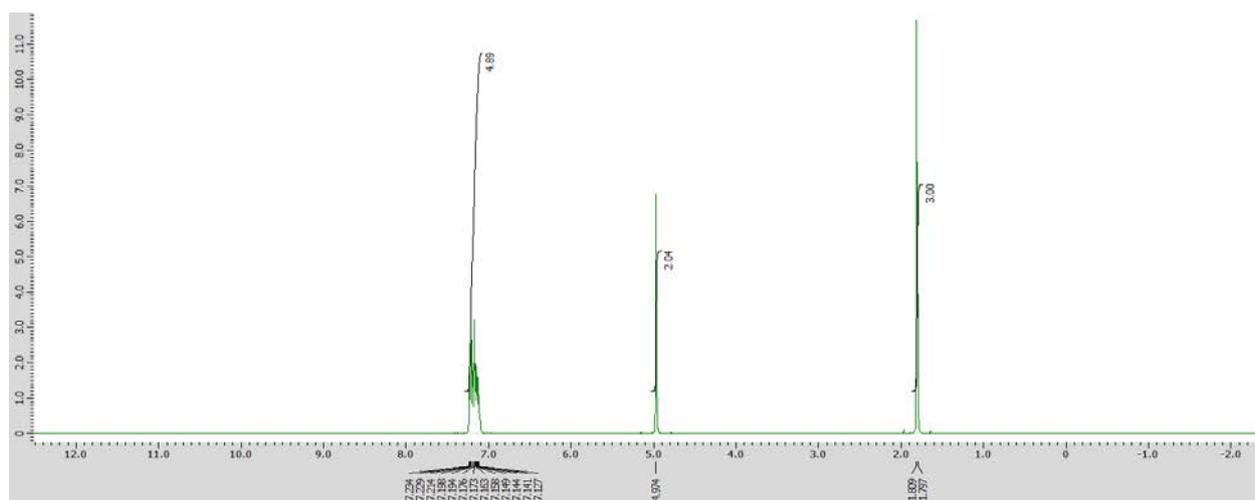


Figure 1. NMR spectrum of >99% anhydrous benzyl acetate

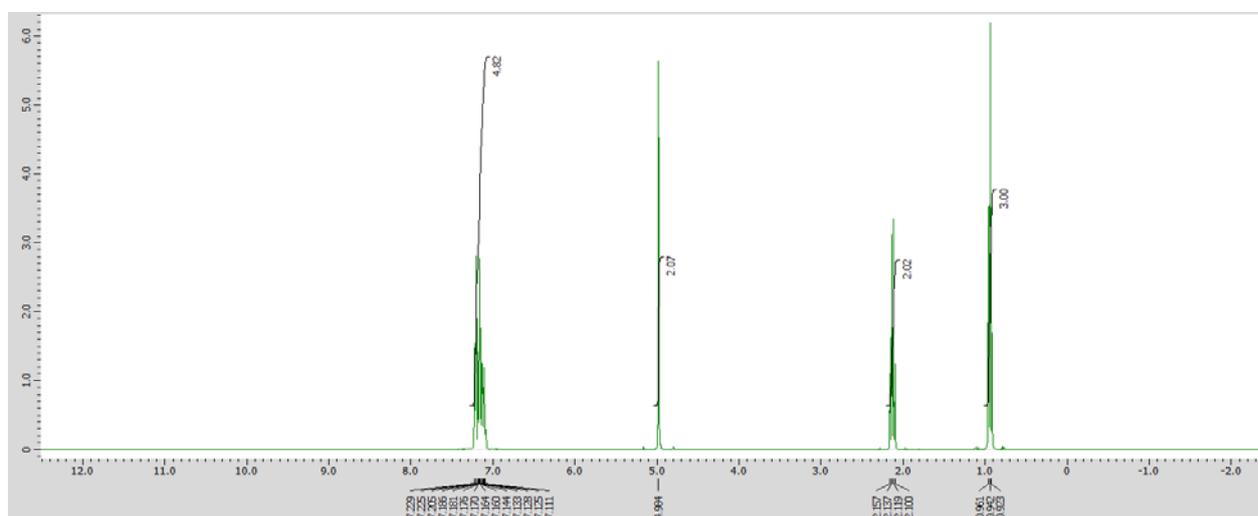


Figure 2. NMR spectrum of >99% anhydrous benzyl propionate

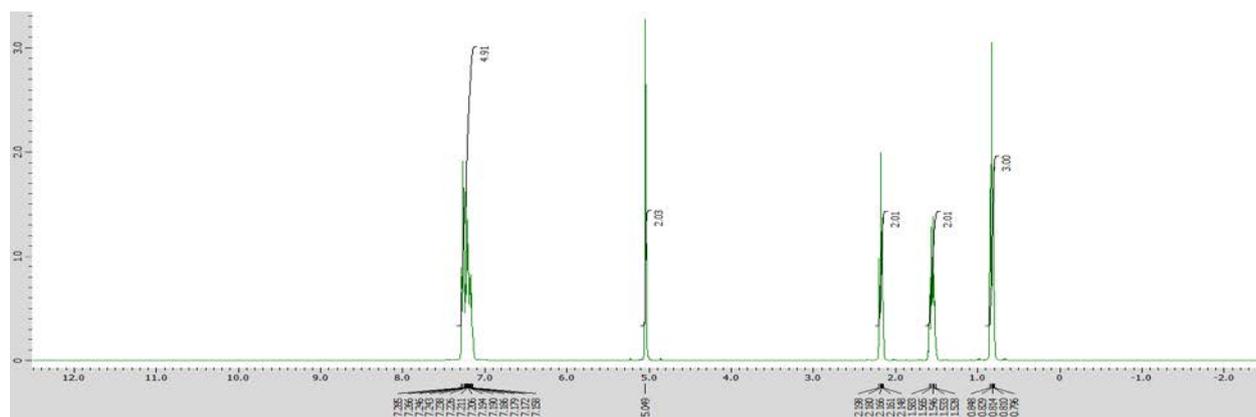


Figure 3. NMR spectrum of >99% anhydrous benzyl butyrate

Table 3. Summary of reagent chemical shift values, multiplicities, and normalized integration values

Benzyl acetate (BA)				Benzyl propionate (BP)				Benzyl butyrate (BB)			
Proton	Shift(δ) ppm	Multiplicity	Integration	Proton	Shift(δ) ppm	Multiplicity	Integration	Proton	Shift(δ) ppm	Multiplicity	Integration
Benzyl	7.30	multiplet	4.89	Benzyl	7.23	multiplet	4.82	Benzyl	7.28	multiplet	4.91
A	4.94	singlet	2.04	A	4.96	singlet	2.07	A	5.05	singlet	2.03
B	1.81	singlet	3.00	B	2.16	quadruplet	2.02	B	2.20	triplet	2.01
				C	0.96	triplet	3.00	C	1.58	multiplet	2.01
								D	0.850	triplet	3.00

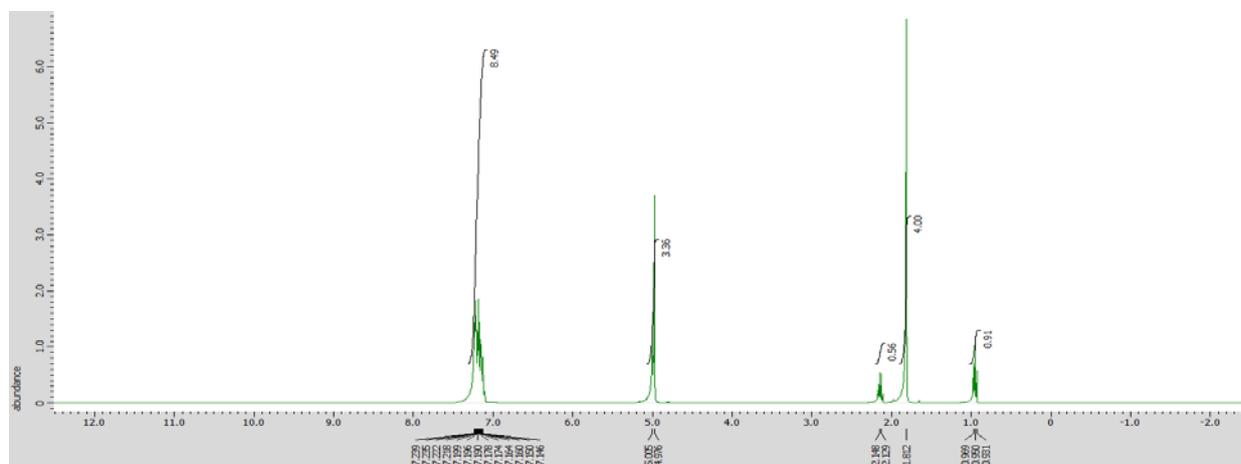


Figure 4. Proton NMR of a 4:1 mixture of BA: BP

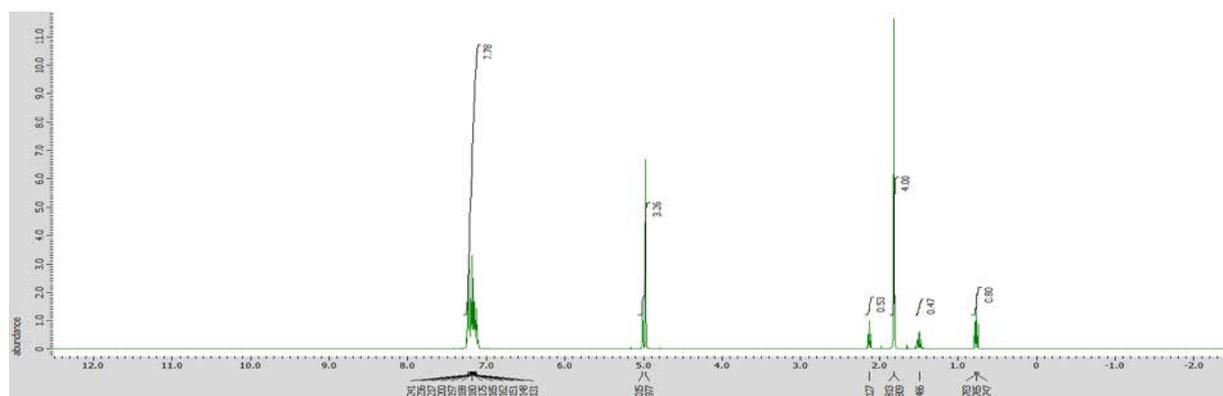


Figure 5. Proton NMR of a 4:1 mixture of BA: BB

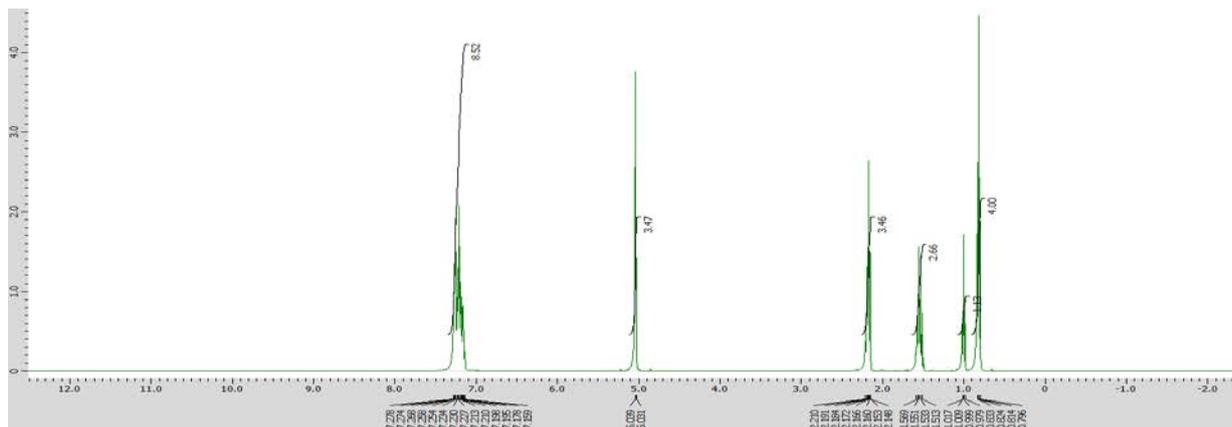


Figure 6. Proton NMR of a 4:1 mixture of BB: BP

Figure 4 through Figure 6 are proton NMR spectra of 4:1 mixtures of BA, BP; BA, BB; and BB, BP respectively. The percent composition of these mixtures was calculated by analyzing the 1.8 ppm singlet peak to quantify the presence of BA, the 0.97 triplet peak to quantify the presence of BP, and the 0.85 triplet peak to quantify the presence of BB. The formula used to calculate this relationship is as follows where BX and BY are the components in the binary mixture in question where A is the number of protons:

$$\% \text{ composition} = \frac{A_{BX}}{A_{BX} + A_{BY}} * 100\%$$

Figure 7 through Figure 9 showcase the plots relating the NMR calculated weight % versus the gravimetrically determined weight % in the absence of TMS and without the 50-50 mixtures as a data point. In all cases, the qNMR methodology is shown to be extremely robust, as all calibration curves have an R^2 value of greater than 0.99.

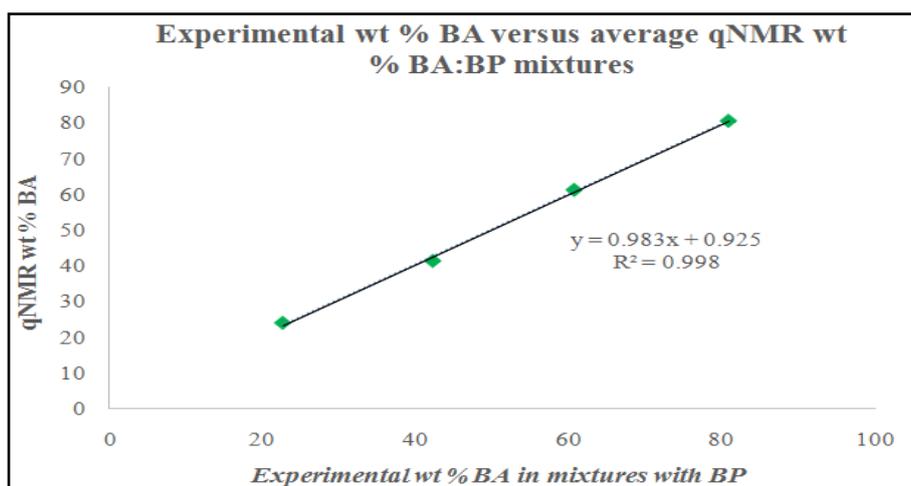


Figure 7. Experimental weight % BA in mixtures with BP plotted against the average qNMR weight % BA without TMS

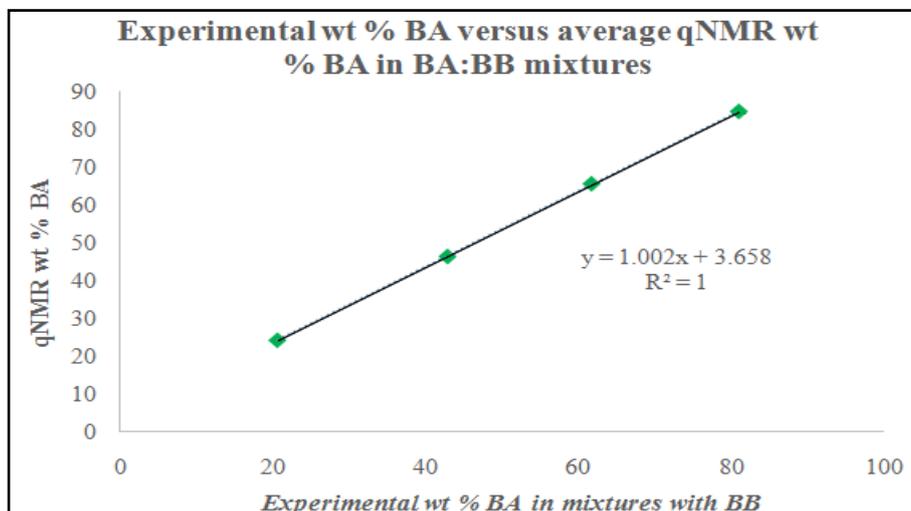


Figure 8. Experimental weight % BA in mixtures with BB plotted versus the average qNMR weight % BA without TMS

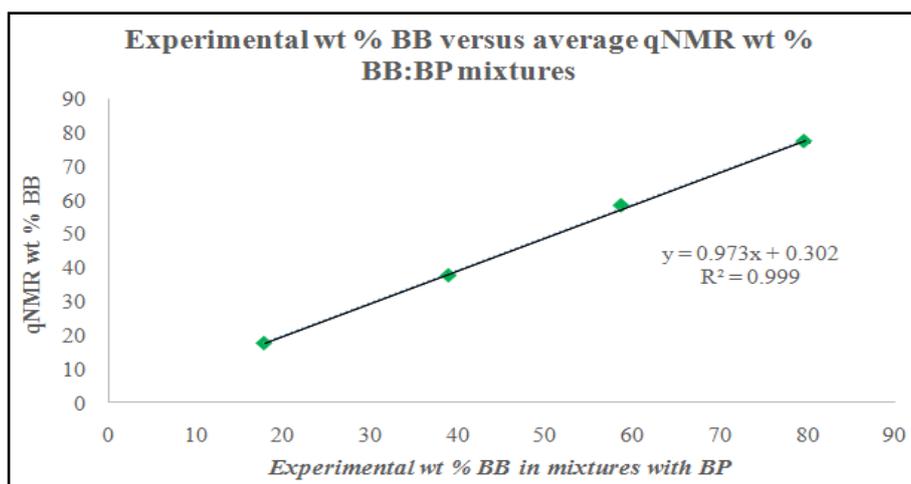


Figure 9. Experimental weight % BB in mixtures with BP plotted versus the average qNMR weight % BB without TMS

In all three cases, there appears to be a one to one correspondence between the experimental weight % obtained via gravimetric analysis and the qNMR weight % since the slopes in all cases are very close to 1. When using the above calibration curves to determine the % composition of the 50-50 mixtures made, the % differences were all below 3%. Absolute differences between the calibration curve's prediction and

the actual weight %'s were even more robust, with the 50-50 mixture having the largest deviation in the BA-BP mixtures with an absolute difference of 1.19 and the best having an absolute difference of 0.609 in the BB-BP 50-50 mixture. The mixtures were also run with TMS as an internal standard and the results are captured in Figure 10 through Figure 12.

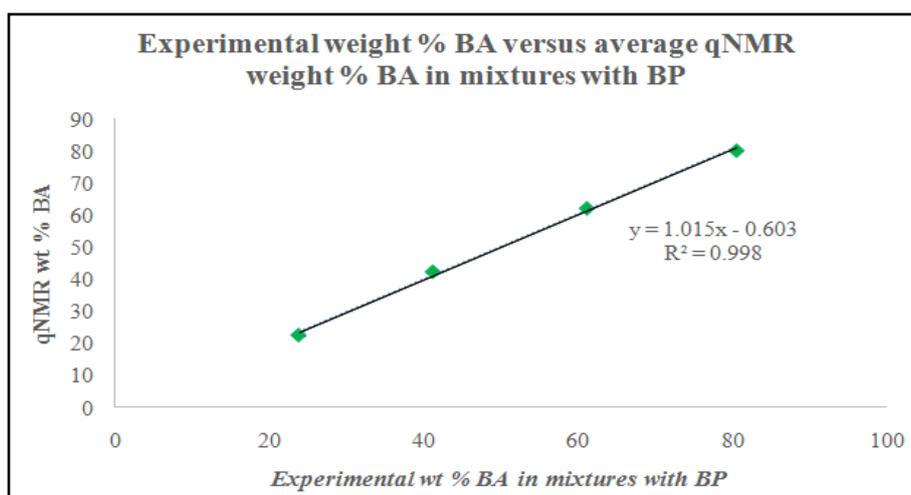


Figure 10. Experimental weight % BA in mixtures with BP plotted against the average qNMR weight % BA with TMS

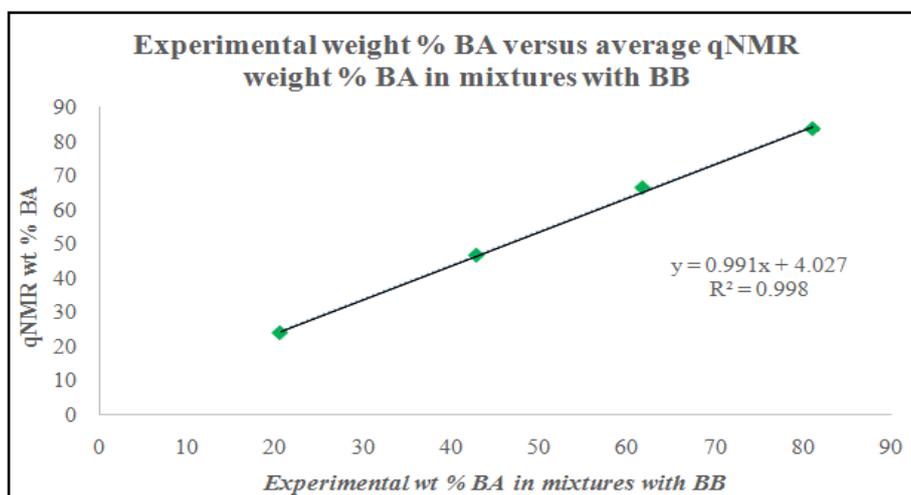


Figure 11. Experimental weight % BA in mixtures with BB plotted versus the average qNMR weight % BA with TMS

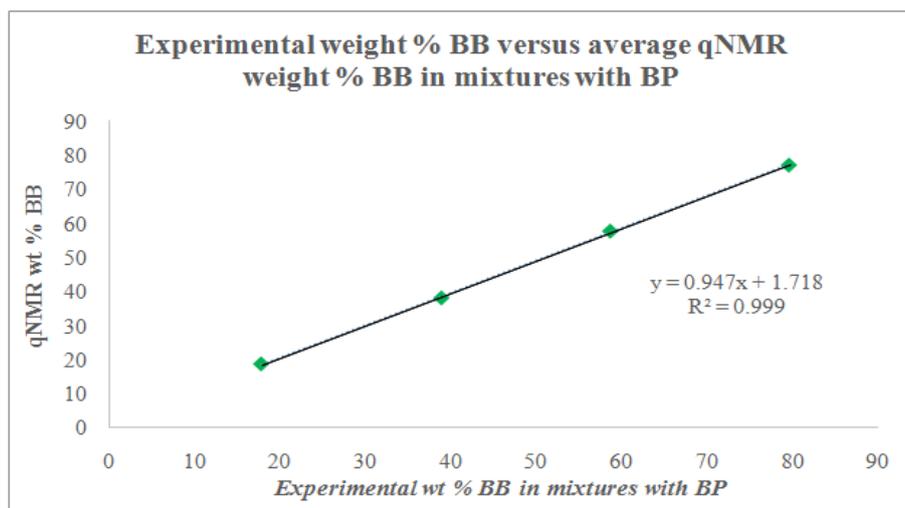


Figure 12. Experimental weight % BB in mixtures with BP plotted versus the average qNMR weight % BB with TMS

4. Conclusions

These calibration curves can be used to quantify, with a great degree of accuracy, the % composition of binary mixtures involving the reagents used in this study. The methodology can be quickly adopted for other experiments using other reagents as this study serves to corroborate previous experiments [5] that proton NMR can be used quantitatively to determine the composition of binary mixtures. There is a direct 1 to 1 relationship between the proton integration values and the gravimetrically determined weight %. This experiment lends support of the quantitative utility of proton NMR at large both with and without an internal reference standard. This experiment serves as an excellent tool for the undergraduate chemistry laboratory.

Acknowledgments

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