Low-field NMR Spectroscopy in Chemical Education

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Abstract Nuclear magnetic resonance (NMR) spectroscopy as one of the most important analytical techniques is a component of almost all chemistry courses. However, the experimental approach is often a particular challenge due to the high costs of the required experimental equipment. The aim of this contribution is to provide NMR experiments suited for practical courses at the undergraduate level and for the education of chemistry teachers. For this purpose, a low-field device was used. A main concern for the chosen experiments was a close relation to relevant everyday life questions of students. The studies comprise experiments around alcoholic fermentation and alcoholic beverages as well as in the field of chemical kinetics. In general, the presented methods yield results in a reasonable range.

Keywords: low-field NMR experiments, alcoholic fermentation, alcohol determination in beverages, chemical kinetics, activation energy

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1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful tools for probing molecular structures and is used across many disciplines. Typical applications in chemistry are, e.g., structure elucidation of compounds, purity determination, process control, monitoring chemical reactions, and determination of dynamic information on molecules. Consequently, NMR spectroscopy has become an integral part of undergraduate curricula in chemistry over the past thirty years [1]. In lectures, NMR is usually introduced by covering topics like fundamental concepts, theory, practical aspects, and application fields of this spectroscopic technique. This way, undergraduate curricula are increasingly taking advantage of NMR techniques to provide opportunities to create significant learning experiences for students. Otherwise, especially laboratory experiments and hands-on activities are well-known to enhance learners teaching engagement, and motivation. However, personal experiences in self-employed recording and interpreting NMR spectra are scarce for many students. Reasons may be, that the sophisticated high-field NMR spectrometers are quite expensive to purchase, operate and maintain, and the high-capacity utilization of these spectrometers in research. As a comparatively less costly alternative for practical courses, educators can use low-field compact benchtop NMR spectrometers (40 – 90 MHz), which are more appropriate for teaching laboratories (cf., e.g. [2]–[4]). One advantage of such types of spectrometers for practical courses is that they are conducted in cryogen-free cooled permanent magnets instead of super-conducted high-field magnets. Another one is the skill to record samples in nondeuterated solvents without additional solvent suppression methods or detector saturation. In terms of linewidth, today's compact low-field NMR spectrometers with permanent magnets can provide resolution similar to that of high-field spectrometers with super-conducting magnets. Of course, the smaller frequency range of benchtop spectrometers leads to lower resolution in the final spectra.

There exist several approaches to implement NMR spectroscopy in the undergraduate curriculum. One can find entire books about this topic, for example, those by Rovnayk and Stockland [5] and Soulsby et al. [1]. In their collections, different authors describe one-dimensional (1D) ^{1}H-NMR experiments for organic or physical chemistry labs but also offer options to teach two-dimensional techniques, complex pulse sequences, or the use of computer simulations. The approaches of Esselman and Mencer are of special interest as they describe the implementation of NMR spectroscopy in high school chemistry courses [6]. Besides those books, Leiren et al. applied NMR spectroscopy as an analytical technique for reaction controlling and determination of the yield of the synthesis of acetylsalicylic acid in an organic chemistry lab for undergraduate students [7].

One of the main challenges in how to introduce this NMR technique in meaningful ways is that at the undergraduate level, students of various programs often attend the same practical courses. In the light of experience, prospective researchers are more interested in a deeper understanding of the theoretical background and experimental techniques of the method. However, prospective teachers are rather interested in best practice applications and explanations. Concerning the interest and self-concept of future teachers, context-based learning has proven to be a promising way to overcome such

challenges [8,9]. Within this framework, it is crucially important to connect the hands-on experiments, chemical phenomena, theories, and representations to relevant contexts for teacher trainees to raise their motivation and promote their interests. In the field of organic chemistry at high school level, alcohols are part of all curricula. In addition, ethanol is especially known to arouse intrinsic motivation in learners [10]. Therefore, in the first step, we focus on experiments to introduce the ^{1}H-NMR technique on a phenomenological basis in connection with the contexts of alcoholic fermentation and alcoholic beverages. This way students are encouraged to obtain their own spectra and perform their own data evaluation to understand the technique. The advantage of this approach is that data acquisition and interpretation are not separate events. Familiar with the basics, we expand in a second step the use of ^{1}H-NMR spectroscopy as a tool for investigating chemical kinetics as one of the main branches of physical chemistry.

2. Alcoholic Fermentation and Alcoholic Beverages

In this section, we present ^{1}H-NMR experiments for benchtop low-field NMR spectrometers, suited for use in practical courses for future teachers. Therefore, these experiments had to be optimized for the application of low-field spectrometers and they should be informative and meaningful for the students and their subsequent profession. Context-based experiments – like those which use reactions known from everyday life – have the potential of catching the student's interest [11]. Only a few published experiments could be found that focus on contextualized experiments. Some experiments that focus on fermentation and the determination of alcohol content in beverages [12] and in alcoholic mixed drinks [13] were already described. Furthermore, Bonjour et al. analyzed "rubbing" alcohol [2]. We focused on the fermentation reaction because it is a pleasant and suitable combination of everyday life chemistry. For the use in practical courses, this experiment was optimized. In addition, different methods were applied to determine the alcoholic content. These and similar analytical techniques can also be utilized on other alcoholic beverages like liqueurs and spirits. In this context, the fundamental principle for using ^{1}H-NMR spectroscopy for quantitative analysis is the proportional correlation of concentration and the signal intensities. More specifically, the signal intensity I_X of a substance X is proportional to the number of nuclei N_X and the amount of substance n_X, that generates the analyzed signal. The constant k of proportionality in Eq. 1 is specific for the used NMR spectrometer and the measuring conditions [14].

$$I_X = k \cdot n_X \cdot N_X \qquad (1)$$

The investigation of alcoholic fermentation provides an intuitive approach to this basic concept of quantitative NMR.

2.1. Investigations of Alcoholic Fermentation

In many German regions there exists a culture of producing wine or beer via alcoholic fermentation. Moreover, this (stoichiometric) simple biochemical reaction is part of the chemistry curricula in secondary schools (e.g. in Bavaria [15]). We selected the fermentation of apple juice as an example, typical for the region Hessen/ Baden-Württemberg and like producing cider. The fermentation process, i.e., the conversion of sugar to ethanol, can be monitored with NMR measurement [12]. We optimized this fermentation with baker's yeast to be carried out within three hours.

2.1.1. *Material and Methods*

For this reaction, 20 g of baker's yeast was dissolved in 25 mL apple juice (freshly pressed) in a 150 mL beaker and mixed with a stirring rod. In a 500 mL three-neck flask with a thermometer and fermentation lock another 125 mL apple juice was heated up to 40 °C. The experimental setup is shown in Figure 1. The yeast and apple juice mixture were added, and the time measurement was started. After each 10 minutes 3 mL of the solution was extracted and centrifuged at 3000 rpm for 20 seconds. 500 µL of the centrifuged solution was then transferred into a 5 mm NMR tube and an NMR spectrum was recorded. The residue was returned into the flask. After a reaction time of 140 minutes the fermentation process was finished.
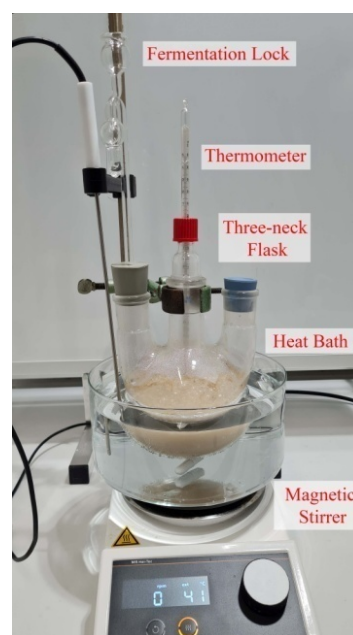

Figure 1. Experimental setup for the fermentation process

All ^{1}H-NMR spectra have been acquired with a Spinsolve 60 MHz benchtop NMR spectrometer (Magritek, Aachen, Germany). A one-pulse sequence, available in the control program Spinsolve, with a pulse width of 90° and 4 scans has been used. The processing (Fourier transformation, line broadening, phase correction, baseline correction) and the spectra analysis have been carried out with the Software TopSpin 4.2 (Bruker, Billerica, MA, USA). The relaxation time T_1 of the CH$_3$-signals of ethanol has been determined with an inversion

recovery pulse sequence to $T_1 = 4$ s. All spectra have been calibrated to the water peak at $\delta = 4.79$ ppm [16].

For quantitative determination of the growing alcoholic content in the reaction mixture, a method well-known for analyzing binary mixtures with high-field spectrometers [14,17] was employed. For low-field spectrometers, the method also proved suitable for determining the alcohol content of spirits [18]. Due to the sugar in the apple juice and its signals, this approach must be slightly adapted. It uses the relative signal intensities of water and the signal of the protons in the methyl group of ethanol and doesn't need an internal standard added to the solution. Based on Eq. 1 the alcohol content can be calculated as a weight fraction by:

$$\frac{m_{EtOH}}{m_{H_2O} + m_{EtOH}} = \frac{\dfrac{I_{CH_3}}{3} \cdot M_{EtOH}}{\dfrac{I_{H_2O} - \dfrac{I_{CH_3}}{3}}{2} \cdot M_{H_2O} + \dfrac{I_{CH_3}}{3} \cdot M_{EtOH}} \quad (2)$$

Herein m stand for the masses of ethanol and water, respectively. M are the molar masses and I stand for the signal intensities. The assignement of NMR signals is illustrated in Figure 2. The signals of the protons in the methyl group of ethanol (green) and the common signal at $\delta = 4.79$ ppm (with intensity I_{H_2O}) of the protons in water and the hydroxy group of ethanol (red) are highlighted.
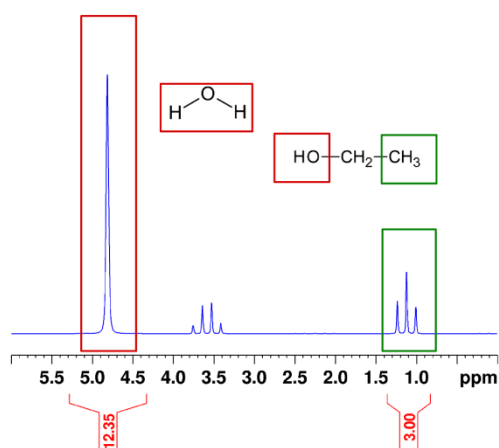

Figure 2. Determination of the ethanol concentration as a weight fraction in a sample of vodka by comparing signal intensities [3]

This method has been calibrated with samples of known ethanol concentration and the calibration curve gave a correction factor of 1.03. The content of alcohol (i.e., ethanol) by volume (ABV) is then calculated out of the weight fractions using data from literature [19] and therefore taking into account the volume contraction. As the last point, the integrals must be set appropriately. That means, on the one hand, they should cover as much signal as possible. On the other hand, they shouldn't overlap with other signals. This is true for all quantitative experiments in this contribution. In this reaction, the limits

for the integrals are for I_{H_2O} $\delta = 4.1 - 5.5$ ppm and for I_{CH_3} $\delta = 0.8 - 1.5$ ppm.

2.1.2. Results and Discussion

The recorded NMR spectra and the observable signals change steadily as the fermentation process continues. The combination of the spectra in Figure 3, recorded over a period of two hours, provides an intuitive way of monitoring the fermentation reaction. In apple juice, the signals of sugars (i.e., glucose, fructose, and sucrose) are observable ($\delta = 3.3 - 4.2$ ppm) besides the strong water peak at $\delta = 4.79$ ppm. In the following spectra, the intensity of the sugar signals decreases while the intensity of the signals of ethanol increases (quartet of the methylene-group at $\delta = 3.65$ ppm and the triplet of the methyl-group at $\delta = 1.17$ ppm). Especially the signal of the methyl protons is a good indicator of the forming ethanol. In these optimized circumstances, the fermentation reaction is completed within about two hours, which can be seen as the signal intensities staying at a constant value.
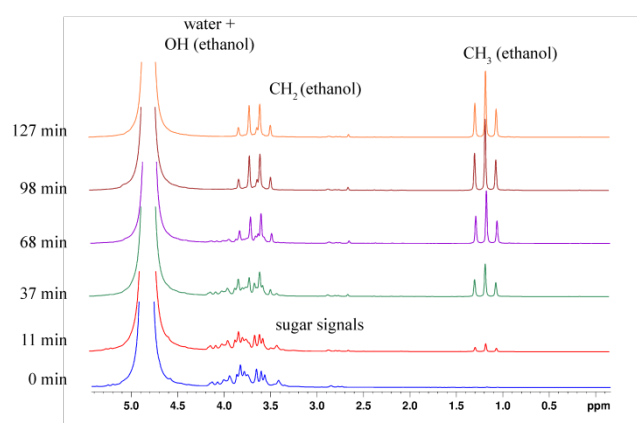

Figure 3. Selected ^{1}H-NMR spectra (60 MHz) of the fermenting apple juice with signal assignments

With the described method the content of ethanol as alcohol by volume was determined for every measured spectrum. In Figure 4 the calculated ABV-values are presented in dependence on time. As can been seen, the alcohol (i.e., ethanol) content increases over time until it reaches a value of about 5.4%. This is verified by an additional measurement after 260 minutes.
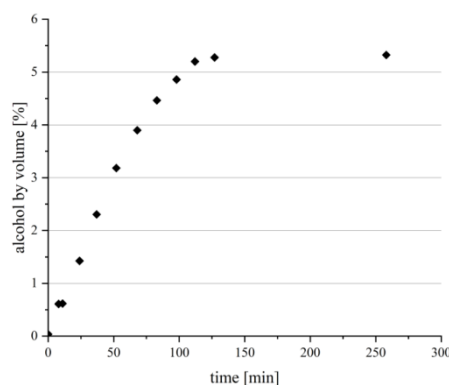

Figure 4. Progress of the fermentation reaction of apple juice

The fermentation reaction is a well-suited example for a simple and well know reaction that can be studied with NMR measurements. Furthermore, the used method is characterized by a low preparative effort and a fast and reliable evaluation that leads to few errors. Therefore, and due to the relatively short reaction time this experiment suits the constraints of a practical course at university. Without yeast additive, the fermentation needs about 500 hours at room temperature, making the experiment infeasible in practical courses. Besides that, the monitoring and analyzing of this reaction can be automized by special monitoring kits as described in section 3.2.2.

2.2. Analyzing Liqueurs and Spirits

Ethanol is only one product of fermentation processes of fruits, their juices, and mashes. Further enzymatic reactions produce other alcohols like methanol, which is even more poisonous. It is formed due to the decomposition of methyl esters in pectins, which are proteins of fruit skins. Therefore, methanol is always a side product of fermentation of fruit-based spirits and wines. Despite multiple distillation steps, some spirits can contain up to 0.8% methanol [20]. While fermented juices like the one in section 2.1 contain too small amounts of methanol for determination with a low-field NMR spectrometer, commercially available spirts are well-suited for this analytical approach. Only fruit-based spirits like fruit brandy contain enough methanol to detect it. Other spirits on the base of potatoes or grain, like whiskey or vodka, contain almost no methanol (at least under 0.05% according to EU-law [21]). Hence, methanol containing vodka or whiskey are always the product of contamination, may it be intentionally or unintentionally. Reports on casualties [22] due to methanol contaminated spirits are therefore an interesting context for analyzing spirits and liqueurs with NMR spectroscopy.

The method for binary mixtures that was used in section 2.1 has already been applied for analyzing alcoholic beverages with high-field NMR spectrometers [17]. Isaac-Lam even used a low-field NMR spectrometer with internal and external standards to investigate the ethanol content in commercially available spirits [23]. We used internal standards and the method for binary mixtures as well with a low-field spectrometer and compared the obtained values with those from a high-field NMR measurement and the manufacturer specifications. Furthermore, the methanol content in fruit-based spirits was investigated by low-field NMR. The results were compared with values that were measured with a high-field spectrometer and by gas chromatography. While most spirits are almost binary mixtures of water and ethanol (and in some cases a quantity of methanol), liqueurs contain a remarkable amount of sugar. The same is true for special fruit wine products like concentrates of apple sherry. As an additional experiment, NMR spectroscopy was used to determine the sugar and ethanol content of such a probe.

2.2.1. Material and Methods

A major advantage of NMR spectroscopy is the simultaneous determination of ethanol, methanol, and sugar with only one measurement. The ethanol and methanol content were analyzed in six different fruit-based spirits that were bought in a local supermarket. Additionally, the ethanol and sugar content of a concentrate of apple cider from a local winemaker was determined. For the measurement that used an internal standard, samples had to be prepared by adding 0.116 g (1 mmol) maleic acid (MA, analytical grad) as the internal standard to 5 mL of the spirit (c(MA) = 0.2 mol). 500 µL of the samples were then transferred into a standard 5 mm NMR tube and spectra were recorded. For all analyzed samples the T_1-values were smaller than 2 s. For low-field measurements three samples have been prepared and analyzed. A one-pulse sequence with a pulse width of 90° and 16 scans (6.4 s acquisition time) has been used. For calibration, high-field ^{1}H-NMR spectra (400 MHz Bruker Avance III Nanobay) were acquired. For frequency lock d_6-Acetone in a capillary was used. The spectra were aligned by shifting the signal of maleic acid to 6.30 ppm.

For ethanol determination, the same method as in section 2.1 can be applied as spirits and liqueurs are basically binary mixtures (BM) of water and ethanol. Another established method for quantitative NMR measurements is the use of an internal standard [23]. Based on Eq. 1 the unknown concentration *c* of the substance *X* (in this case ethanol) can be calculated with the concentration of the internal standard *S* and the signal intensities *I* of *X* and *S* and the number *N* of protons that generate the specific signal as

$$\frac{c_X}{c_S} = \frac{I_X}{I_S} \cdot \frac{N_S}{N_X} \Leftrightarrow c_X = \frac{I_X}{I_S} \cdot \frac{N_S}{N_X} \cdot c_S \tag{3}$$
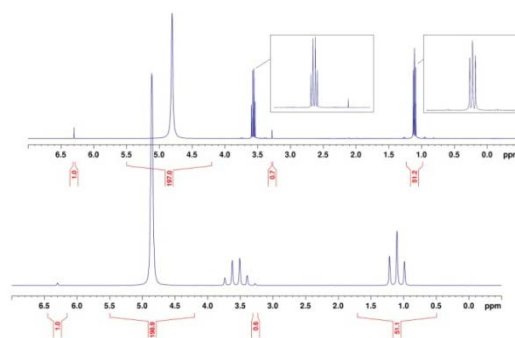

Figure 5. Comparison of a 400 MHz (above) and a 60 MHz (below) ^{1}H-NMR-spectrum of a Mirabelle plum brandy that contains maleic acid as an internal standard. The integrals show the intensities of the signals of the protons of maleic acid (δ = 6.3 ppm; vinyl protons), water (and the hydroxy groups of the alcoholic compounds at δ = 4.8 ppm), the methyl group of methanol (δ = 3.3 ppm) and the methyl group of ethanol (δ = 1.1 ppm) [3].

For the determination of ethanol concentration, the signal intensity of the triplet of ethanol has been used. Figure 5 shows the integrals of signals of ethanol, methanol, and maleic acid recorded in a high-field

and low-field NMR spectrometer, respectively. The ^{13}C-satellites were not integrated due to overlap with other signals and their relatively great distance to the main peak. To consider this, a calibration with samples of known concentrations of ethanol and methanol was performed. The calibration for ethanol lead to a correction factor of 1.05.

The determination of methanol concentration with an internal standard is carried out in the same way as for ethanol. The protons in the methyl group of methanol give a singlet peak at 3.3 ppm (cf. Figure 5). In the low-field spectra this signal overlaps with the signal of the methylene protons in ethanol to a small extent and the integral of the methanol signal must be observed with a linear baseline correction. The following calibration with samples of known concentration of methanol in a water-ethanol solution yielded a linear correction function y = 1.029 x + 0.003. As an alternative, the addition-method was tested for methanol concentration. Anders et al. used this technique in the 1970s for determination of ethanol content [24]. For this method, two samples must be prepared where one is the original sample and to the other one a known quantity of methanol is added. The difference between the signal intensities can then be used to calculate the primal concentration of methanol. This technique yields comparable values as the internal standard but with smaller errors. In addition, the methanol content has also been investigated via gas chromatography for comparison (Shimadzu GC-2010 gas chromatograph with a ZB-WAXplus-column).

The internal standard method (IS) was also used to determine the sugar concentration in the apple cider concentrate. Therefore, it was assumed that the whole sugar in the concentrate was made up of glucose and the various sugar signals in the range of δ = 3.3 – 4.2 ppm were used for integration. The signal of the methylene-protons of ethanol in the same area and some overlap of the water peak must be considerd. The signal intensity of the methylene-protons can be simply substracted. It is calculated by measuring the intensity of the methyl-protons and multiplication of this value with the factor 2/3. The residual signal of the strong water peak must be accepted. Besides that, calibration with samples of a known concentration yielded values with an error smaller than 5%. The error caused by assuming that the whole sugar in the wine concentrate would be glucose, is larger than the calibration error. Hence, no calibration factor is needed in practical courses.

2.2.2. Results and Discussion

The determined values for the ethanol concentration (i.e., the ethanol content as alcohol by volume) are summarized in Table 1. The given values show the alcohol by volume in percent based on measurement via a low-field and a high-field NMR spectrometer compared to the manufacturer specifications.

As can be seen, the method for binary mixtures (BM) yields more precise values. This can easily be explained as there is no sample preparation for this case, so the only possible errors result from the measurement itself (e.g., from a bad shimming of the spectrometer) or the wrong measurement of the signal intensities. For methods that are used to analyze foods there exist strict guidelines and

standards. According to EU-law a deviation of 0.3% (ABV) is allowed for the declaration of the alcohol content [25]. This could not always be achieved with the presented method, but it fits the requirements of understanding the principles of quantitative NMR in terms of an everyday life problem.

In Table 2 the results of the determination of the methanol content are summarized and compared to the values of the measurement with a high-field spectrometer and a gas chromatograph. Besides that, the limits for methanol in the respective spirit according to EU-law [21] are indicated.

Table 1. Results of the Determination of Ethanol Content in Different Liquors by ^{1}H-NMR Spectroscopy

Spirituous Liquor	60 MHz IS	60 MHz BM	400 MHz IS	Manufacturer specifications
Grappa	40.6 ± 0.5	39.9 ± 0.3	39.6	40
Mirabelle plum brandy	41.0 ± 0.8	40.6 ± 0.4	39.2	40
Tequila	39.0 ± 0.4	38.5 ± 0.1	37.0	38
Fruit brandy	38.9 ± 1.6	38.9 ± 1.3	37.8	38
Plum brandy	40.1 ± 0.4	40.1 ± 0.1	39.1	40
Pear brandy	40.5 ± 0.6	40.2 ± 0.1	38.4	40
Apple cider concentrate	21.5 ± 0.4	20.8 ± 0.2	20.1	-

Table 2. Results of the Determination of the Methanol Content in Different Liquors in Gram per Hectoliter Based on the Total Alcohol Content

Spirituous Liquor	60 MHz NMR	400 MHz NMR	Gas chromatography	EU limit value
Grappa	410 ± 9	464	427 ± 14	1200
Mirabelle plum brandy	683 ± 16	748	709 ± 15	1200
Tequila	156 ± 2	152	163 ± 5	-
Fruit brandy	619 ± 6	693	615 ± 2	1200
Plum brandy	794 ± 10	865	848 ± 8	1200
Pear brandy	897 ± 13	947	921 ± 12	1350

It can be clearly seen that methanol is highest in pear brandy, but all the observed methanol contents are below the allowed limits for every spirit. Besides some experimental errors and deviations, all values are within a reasonable range. The qualitative detection of methanol with the low-field spectrometer is possible for methanol contents over 0.05%. In the apple cider concentrate a small signal for methanol was detected only in the high-field spectrum, yet it was too small for quantification.

The observed sugar content in apple cider concentrate with the low-field spectrometer and maleic acid as an internal standard was 170 g/L. High-field measurement yielded a value of 180 g/L and gravimetric measurement gave a value of 175 g/L. All given values are referred to the molar mass of glucose.

Generally, the presented experiments and methods yield values that are in a reasonable range. Although they are not as precise as other methods in food analytics, they allow an approach of learning the principles of quantitative NMR spectroscopy. These methods are characterized by their small preparation effort and their versatility for many alcoholic beverages. With the presented experiments, students learn about analytical

standards, ways to reference their measurements and think about problems that can arise in the analytical process, like systematic and random errors. They therefore need statistical methods to get around these problems.

3. Experiments for Kinetic Studies

Besides usage in undergraduate organic laboratory courses, low-field NMR spectrometers also provide a convenient way to introduce teacher trainees into the principles of reaction kinetics. The area of chemical kinetics as one of the main branches of physical chemistry is generally concerned with studying the speed or rate of chemical reactions under various conditions and interpreting these data to obtain general information about the mechanisms of chemical reactions and transition states. To calculate reaction rates for slower reactions, the concentration of a product or reactant over time is mostly observed by classical wet chemical methods. For faster reactions, the measurement of concentrations is usually replaced by techniques detecting physical properties of a product or reactant which are directly proportional to the concentration. Besides well-established methods like light absorption (IR and UV/Vis), conductivity or pH, also NMR signals can be employed. Therefore, the area of a resolved resonance signal has to be determined with respect to time. In this cases, long series of continuous measurements in dependence on time with a lot of experimental data can be automated by using reaction monitoring kits. For this purpose, the kinetic of above-described alcoholic fermentation of apple juice is too complex and because of that unsuitable. Consequently, a classic first order reaction with well-resolved ^{1}H-NMR resonances of the product or reactant has been chosen as an example.

3.1. A Classic First Order Reaction: The Hydrolysis of 2-Chloro-2-methylpropane (*tert*-Butyl chloride)

The reaction of *tert*-Butyl halogenides with a nucleophile like water is a prototypical example for a S_N1 reaction. Hence, the first order reaction of *tert*-Butyl chloride with water (cf. Figure 6) is well-studied and widely used as an introduction to kinetic studies in practical courses, even for high schools [26]. While the classic experiment uses conductometric measurements, this reaction was adapted for the use of ^{1}H-NMR spectroscopy. To determine the empiric activation energy, the reaction has been performed at different temperatures (10°C, 20°C, and 30°C). The reaction rate depends on the polarity of the solvent. Adding less-polar solvents like acetone slows the reaction rate down so it can be pursued via NMR spectroscopy.
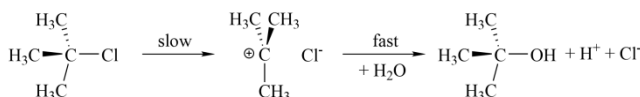

Figure 6. S_N1-mechanism for the reaction of tert-Butyl chloride with water

3.1.1. Material and Methods

For preparation, 0.58 g (5 mmol) maleic acid as an internal standard were added to 50 mL of a water/acetone-mixture (70:30). In another flask, 6.25 mL (57 mmol) *tert*-Butyl chloride were solved in 50 mL acetone. The two solutions were tempered in a water bath. Finally, 1 mL of the solution of *tert*-Butyl chloride was added to the solvent-mixture under stirring and the reaction starts. After 15 seconds the first spectrum was taken traditionally and the whole process was repeated for 15, 25 or 40 min, respectively. It was found, that using one single, tempered probe in an NMR tube yields sufficient results while keeping the effort in practical courses low.

The parameters for the NMR experiments are almost the same as described in section 2. In contrast to these measurements a one pulse-sequence with only one scan has been used due to the fast reaction and the rapidly changing signal intensities. All spectra are aligned to the peak of maleic acid at $\delta = 6.30$ ppm. The signals of the methyl-protons of *tert*-Butyl chloride ($\delta = 1.51$ ppm) and the product *tert*-Butyl alcohol (at $\delta = 1.14$ ppm) are located between the strong acetone signal ($\delta = 2.12$ ppm) and its right-hand ^{13}C satellite (at $\delta = 1.09$ ppm). Only the signal of *tert*-Butyl chloride is used for further evaluation.
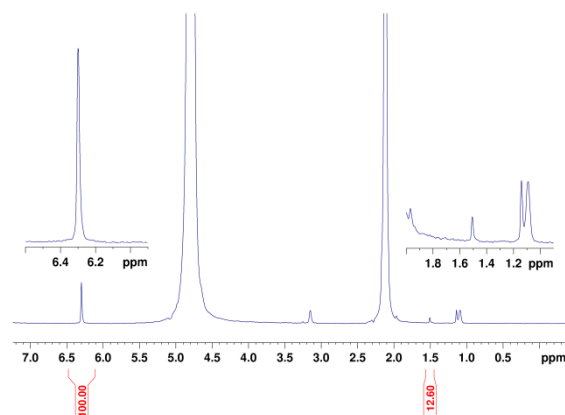

Figure 7. ^{1}H-NMR spectrum of the reaction mixture, 29 minutes after the start of the reaction at 10°C

Adding maleic acid as an internal standard has been chosen to normalize the signal intensities of the signal of the methyl-protons of *tert*-Butyl chloride by setting the signal intensity of maleic acid to 100. The integrated rate law for first order reaction (Eq. 4) was used to determine the reaction rate constant k. In Eq. 4 c and c_0 stand for the concentration and the initial concentration of *tert*-Butyl chloride, respectively, and t is the time in seconds.

$$c(t) = c_0 \cdot \exp(-kt) \qquad (4)$$

As the signal intensity of the methyl peak of *tert*-Butyl chloride is proportional to its concentration, the later one can be substituted by the integral value I of the methyl-proton signal of the reactant.

$$I(t) = I_0 \cdot \exp(-kt) \qquad (5)$$

The obtained $I(t)$ data are plotted against time (Figure 8). The exponential fit yields for 20°C a function of

$$f(x) = 3.42 + 52.59 \cdot \exp(-0.0021x)$$ (R² = 0.98) and therefore, $k = 0.0021 s^{-1}$.
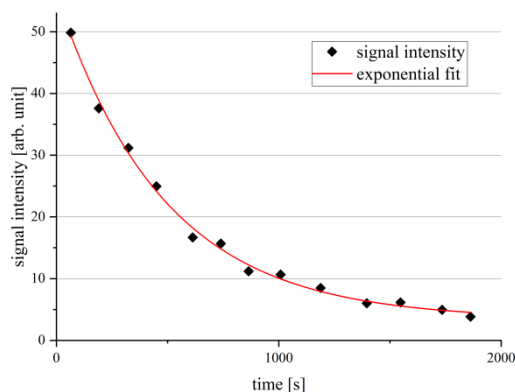

Figure 8. Obtained signal intensities of the methyl-protons of tert-Butyl chloride in dependence on time for the reaction at 20°C

The reaction has been repeated three times at different temperatures ($283, 293$ and $303K$). The obtained values for the rate constants k at these temperatures were taken to determine the activation energy E_A by using the linearized Arrhenius equation (Eq. 6) with Arrhenius parameter A and the gas constant R.

$$\ln(k) = -\frac{E_A}{R} \cdot \frac{1}{T} + \ln(A) \qquad (6)$$

3.1.2. *Results and Discussion*

The calculated averaged rate constants at different temperatures are $11 \cdot 10^{-4}$ s^{-1} (283 K), $21 \cdot 10^{-4}$ s^{-1} (293 K), and $59 \cdot 10^{-4}$ s^{-1} (303 K). For the used solvent mixture, a value of $k = 23 \cdot 10^{-4}$ s^{-1} for 293.5 K was presented in the literature [26], in good agreement with the values above.

According to Eq. 6, these values gave an activation energy of $E_A = 66.1$ kJ/mol. A comparison with literature data is difficult due to the adapted solvent mixture. The activation energies, presented in the literature for this reaction cover a wide range from 59.5 [27] to 96.9 kJ/mol [28]. Therefore, for comparison conductivity measurements were performed, according to a literature procedure [26]. They yielded a value of 72.0 kJ/mol, which is in reasonable agreement with the NMR results. However, specific problems that are inherent for the NMR technique are the signals of acetone, that partially superimpose the investigated peaks. Furthermore, this method is rather time consuming, as every single spectrum should be manually adjusted after automatic processing. Nevertheless, it enables students to understand the principles and limitations of quantitative NMR spectroscopy as an analytical technique for kinetic studies due to the simple reaction and the basic methods that were used.

3.2 Further Investigation on Kinetics: Variations of the Same Experiment

To face the mentioned problems the same experiment has been performed under adapted conditions. To avoid the problems of the solvent peaks overlapping the investigated signals, deuterated water and acetone have been used. Furthermore, in another experiment an automatization kit for reaction monitoring was tested to reduce the effort of recording and analyzing the single measurements.

3.2.1. Use of Deuterated Solvents

It is a distinct advantage of the used NMR spectrometer, that it works with samples in non-deuterated solvent as it can be manually shimmed and locked. In high-field spectrometers deuterated solvents are used for the shim- and lock-process and due to a better receiver gain. Furthermore, overlapping of dominant solvent peaks with interesting signals is a main reason for the use of deuterated solvents. Therefore, the hydrolysis of *tert*-Butyl chloride has also been performed using a 70:30 mixture of D_2O and aceton-d$_6$. Due to the high costs of these solvents, the approach is not foreseen for practical courses. For this experiment, 0.023 g (0.2 mmol) maleic acid were solved in 1.4 mL D_2O and 10µL *tert*-Butyl chloride were solved in 0.6 mL acetone-d$_6$. After tempering both solutions, they were mixed and spectra were recorded at 10°C, 23°C, and 30°C.

In Figure 9 the blue spectrum shows a ^{1}H-NMR spectrum of the reaction mixture in deuterated solvents compared to the red spectrum in protonated solvents. As can be seen, the interesting signals of maleic acid (δ = 6.30 ppm), *tert*-Butyl chloride (δ = 1.51 ppm) and *tert*-Butyl alcohol (δ = 1.14 ppm) are better resolved compared to the much smaller residual solvent peaks of HDO (δ = 4.79 ppm) and acetone-d$_5$ (δ = 2.1 ppm). Therefore, the further evaluation and the determination of the signal intensities does not suffer overlapping and bad resolved signals using deuterated solvents.
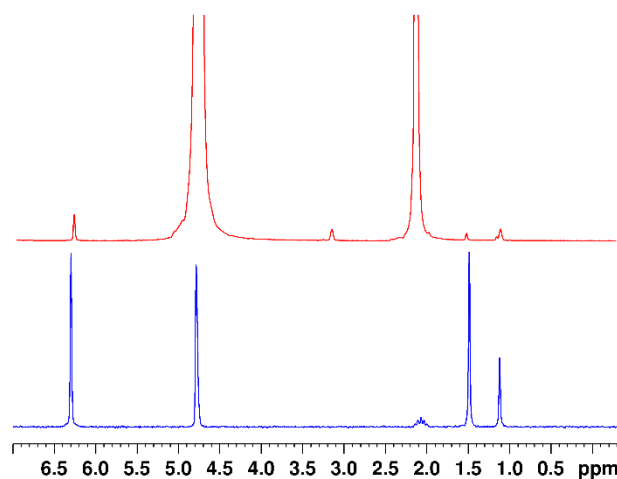

Figure 9. Comparison of ^{1}H-NMR-spectra of the reaction mixture in protonated (red spectrum) and deuterated (blue spectrum) solvent mixtures

The obtained values for the rate constants are much smaller which corresponds to longer reaction times. A possible explanation is the slower motion of deuterated species (cf. motion of molecules and their corresponds to activation energy according to the collision theory). The Arrhenius equation yielded an activation energy $E_A = 87.6 \pm 6.4$ kJ/mol, which is appropriately a little higher than the values obtained in protonated solvents.

3.2.2. Automatization in Kinetic Studies: NMR Reaction Monitoring Kits

Manual sampling, recording, and analyzing are quite time consuming. For automatization, reaction monitoring kits are available, which are basically made up of a glass flow cell for the spectrometer, a peristaltic pump and Teflon tubing. Using this kit, it is possible, to pump a reaction mixture continuously or in a stopped-flow mode through the glass flow cell into the spectrometer and record NMR spectra automatically. Afterwards, the reaction mixture is pumped back into the reaction flask. The results, using such a feature, are presented in Figure 10. The spectra show a change in intensities of the characteristic signals of reactant and product (signed in the figure) as the reaction proceeds.
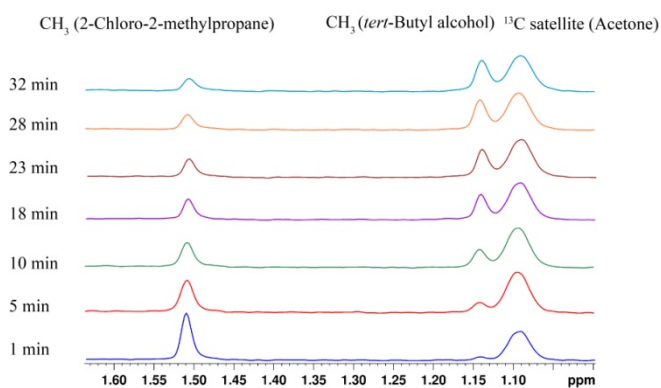

Figure 10. ^{1}H-NMR spectra over time of the hydrolysis of 2-Chloro-2-methylpropane at 10 °C, recorded via a reaction monitoring kit

From the data at three different temperatures via Arrhenius equation, an activation energy of $E_A = 72.0 \pm 5.1$ kJ/mol was calculated. This result is in the range of the values obtained with single measurements and manual analyzing processes. Therefore, the reaction monitoring kit is perfect for automatization of routine processes. In practical courses its use may be inappropriate as major parts of the analytical process are black boxes. Nevertheless, the use of this kit also allows the monitoring of very slow reactions like the fermentation reaction of section 2.1. under normal conditions.

4. Conclusions

In this contribution various experiments have been presented which illustrate how low-field NMR spectroscopy can be employed in practical courses for students at the undergraduate level and especially for teacher trainees. The basic concept was to design experiments involving ^{1}H-NMR spectroscopy permitting to answer everyday life questions relevant to teacher students. Experiments in the contexts of alcoholic fermentation and alcoholic beverages are well-suited for this purpose. For teaching students, the basic principles of reaction kinetics, experiments with reactions of classic first order with NMR detection were developed. The kinetic studies are reliable and can be interpreted commonly. Currently, the experiments are introduced in undergraduate laboratory courses for teacher trainees at Julius-Maximilians-University Würzburg. The follow-up

evaluation and empirical survey concerning the interest and self-concept of future teachers are in preparation.

Direct access to NMR instrumentation for teachers at high schools seems not to be realistic as the costs are probably too high for high school budgets. However, with the self-gathered experiences of teachers in NMR already during their studies, collaborations with local universities become more likely. For the preparation of students and the introduction into the theoretical background of NMR an interactive platform on a didactical reduced basis is available [29].

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