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CapPack devices for enhanced qNMR measurements in ^{1}H NMR spectroscopy

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CAPPACK DEVICES FOR ENHANCED QNMR MEASUREMENTS IN ^{1}H NMR SPECTROSCOPY

by

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ABSTRACT

Quantitative NMR analyses can be improved by adding well-defined external references for measuring sample properties on absolute scales. Special Capillary-tube Package (CapPack) devices were invented that provide *in situ* information (e.g., temperature, pH, pressure, integration, chemical shift, lock, etc.) about a sample with spectral imprimaturs. A new microscale glass-sealing technique produced CapPack devices that can survive high temperatures and pressures in harsh environments. Two CapPack devices are discussed: (1) Gradient CapPack– a device for examining the irradiation bandwidth of solvent-suppression pulse sequences; (2) T_1 CapPack - a device for examining the T_1 robustness of solvent-suppression pulse sequences. A method of calculating the volume factor of external reference using NMR is also presented.

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1. INTRODUCTION

1.1. BACKGROUND AND REVIEW

Nuclear Magnetic Resonance (NMR) spectroscopy is perhaps one of the most powerful analytical techniques in chemical, biomedical, and pharmaceutical research institutions. It provides the most unambiguous information on structures, conformations, dynamics, and functions of chemicals. [1] NMR is a non-destructive, single-assay technique that does not require sample derivatization or separation columns, and it is particularly useful in recognizing as well as quantifying compounds, such as sugars, amino acids, and other relatively unreactive compounds that are not easily detected or measured by other metabolomics approaches. Because NMR has the ability to identify and quantify hundreds, and potentially thousands of small molecules,[2] quantitative NMR (qNMR) has been developed for identifying and quantifying molecular structures. In comparison with commonly used chromatographic techniques (e.g., High-Performance Liquid Chromatography, HPLC; Gas Chromatography, GC; etc.), qNMR as a structure-enhanced analytical technique can be applied to characterize carbohydrates of bio-drugs, such as heparins, and does not require complex sample separation.

1.1.1. qNMR as a Quantitative Analytical Technique. qNMR stands for "quantitative NMR" and refers to the use of NMR to determine the concentration of one or more chemical species in solution. Conducting qNMR properly and to a very high level of precision takes some special consideration. For example, in one-dimensional experiments without hyperpolarization or polarization transfer from other nuclei, the area of an NMR peak is directly proportional to the number of nuclear spins. Its concentration can easily be deduced by comparison to signals from compounds of known

concentrations, particularly because the NMR "response" will be the same for all molecules. This feature makes qNMR stand ahead of almost all other metrological techniques used to quantify compounds for they usually suffer from problems such as compound-specific response factors (UV), chemical considerations (NCLD), or relative volatility (ELSD).[3]

For qNMR, there is usually no elaborate sample preparation or compound separation needed as in other kinds of spectroscopic techniques. In addition, qNMR offers the structural information of NMR experiments with its uniquely rich qualitative details. It is therefore not surprising that, over the last two decades, qNMR can be found in several fields of research or routine analytical measurements as a basic technique. These fields include accurate determination of the purity levels of active pharmaceutical ingredients (APIs) and drug analyses, [(4-5)] quantitation of natural products [6], quantitation of pharmaceutical compounds [7], forensic analyses [8], and food sciences [9].

1.1.2. Review of Current qNMR Methods. qNMR is gaining interest across both analytical and industrial research applications and has become an essential tool for the content assignment and quantitative determination of impurities. Two aspects must be considered to make an NMR experiment quantitative: (a) an internal or external reference is needed, and (b) data acquisition and processing parameters must be kept constant.

Girardeau's paper[10] gives a brief description of reference techniques, as well as computational methods, such as calibration curves to assist in obtaining quantitative measurements routinely and reproductively. In Santosh and Raja's paper [11], the emphasis is on ^{1}H qNMR. They show that it is critical to detect the proton signal (i.e, ^{1}H signal) on resonance in order to determine the purity of a molecule or a mixture. Walter's paper[12]

presents the importance of using external references for quantitative measurements by ^{1}H NMR. For hydrogen-containing compounds of low molecular weight, a precision and accuracy of about 1% is obtainable with high-field NMR spectroscopy when reference and analytes are placed in separate but identically sealed precision glass NMR tubes. In practice, the overall process of conducting qNMR experiments includes specific selection criteria, pre-tests, experimental conditions, homogeneity and stability studies.

1.1.3. qNMR in Hydrothermal Degradation Reaction. In a sponsored research project, we intended to apply standard qNMR measurements to the intermediates and products of hydrothermal degradation reaction of cellulosic biomass. Hydrothermal degradation is considered a carbon-efficient method to convert readily available cellulosic biomass to the valuable liquid-fuel precursor molecule 2, 5-dimethylfuran (2, 5-DMF), and it is seen as a possible alternative to the currently conducted, less energy-efficient industrial method of converting biomass to ethanol via fermentation. We experimented using an aqueous solution of D-glucose as biomass model substrate with calcium chloride and its polarizing calcium ions as an inorganic-salt catalyst. The current focus of the project is on the optimization of the conversion of D-glucose to D-fructose and subsequently to 5-hydroxymethyl furfural (5-HMF), which is a crucial series of steps in the overall reaction as shown below in Figure 1.1.

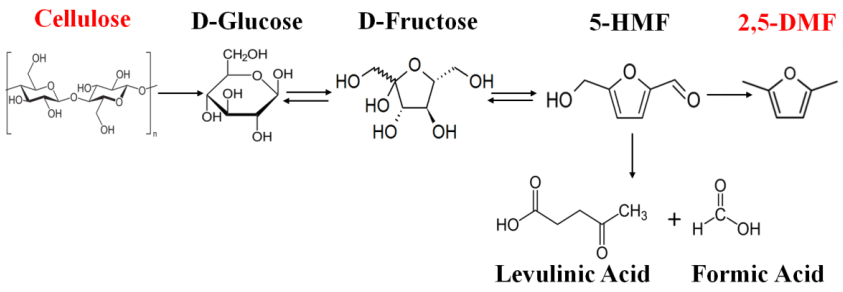

Figure 1.1. Hydrothermal biomass to fuel (HT-BTF) reaction pathway

The reaction that leads to 5-HMF is often acid catalyzed, but an undesired side-reaction will occur if the pH of the reaction is too low (pH < 3). This side reaction will generate levulinic acid (i.e., 4-oxo pentanoic acid) and formic acid. Thus, close pH control appears to be an important aspect for increasing the yield of 5-HMF. Here, qNMR is used to determine the molar concentration and the percentage yield of the product 5-HMF.

1.2. PROBLEM

To measure the degradation products of HT-BTF reactions, NMR was chosen as a superior analytical method. However, for reactions in aqueous solution, the strong water proton signal around 4-5 ppm (depending on the acidity of the solution) is always a problem for NMR measurements, and solvent suppression is frequently necessary for those experiments. For example, most in vivo ^{1}H-NMR analyses of biological samples are performed in aqueous solution with a very high water-proton concentration.[13] Similarly, Figure 1.2 shows the NMR spectrum of a sample taken from the HT-BTF

conversion after 12 hours of reaction at 150 °C. The strong signal in the center of the spectrum at about 5 ppm originates from the water protons of the aqueous solution. This strong water proton signal interferes with the qNMR analysis by integration of the 5-HMF ^{1}H signals (a, b, and c in Figure 1.2), in which we were particularly interested to determine the product yield. The distortion is caused (a) by a partial overlap of the water signal with the 5-HMF signals b and c, and (b) by a distorted baseline of the spectrum. Note that the baseline is severely distorted and will cause serious integration errors for peaks b and c. Therefore, a solvent-suppression NMR pulse sequence was needed to eliminate or, at least substantially reduce the water-signal intensity.

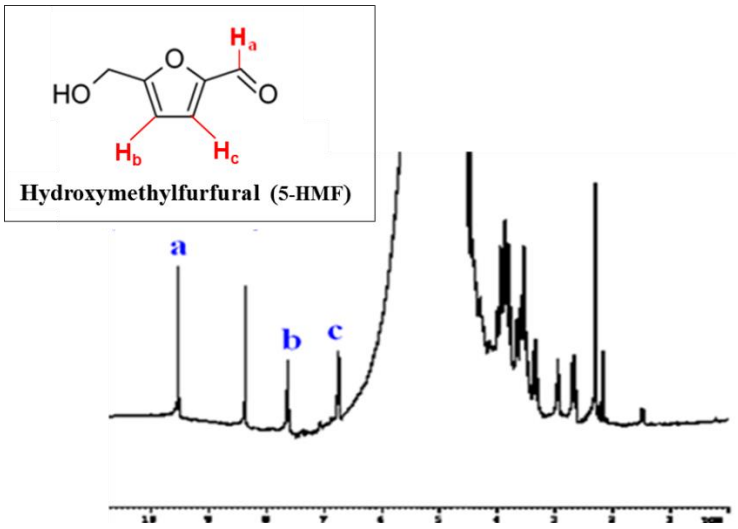

Figure 1.2. ^{1}H NMR spectrum of HT-BTF reaction products without the application of a solvent-suppression pulse sequence. Signals a, b, and c are assigned to the hydrogen atoms at the marked positions of 5-HMF

1.2.1. Solvent Suppression. Dr. Woelk's research group developed a solvent-suppression pulse sequence named EXponentiallly Converging Eradication Pulse Train (EXCEPT), which was tested in this thesis for its effectiveness. EXCEPT consists of a train of 180° pulses with a periodically decreasing interpulse delays. It follows the concept of multiple inversion-recovery nulling and has been computer-optimized to suppress a wide range of T_1 relaxation times. The effectiveness of solvent-suppression pulse sequences such as EXCEPT depends on several factors. Work conducted within the scope of this thesis was aimed at exploring and testing the two most important issues of solvent suppression, which are the suppression bandwidth (i.e., bandwidth distortion factors) and the effects of variations in spin-lattice relaxation on the effectiveness of solvent peak suppression.

1.2.1.1 Distortion factors. The EXCEPT solvent-suppression pulse sequence successfully irradiates solvent signals (water protons in this case) so that it is possible to observe NMR peaks of reaction products in the spectrum. However, the water peak suppression can also distort signals close to the solvent peak and therefore needs to be carefully tested. Accurately measuring distortion factors of signals near the solvent peak is necessary for the purpose of accurate qNMR determination. CapPack devices offer a convenient and time-efficient method to determine the signal distortion over a wide range of NMR signals including the area of the suppressed solvent signal.

1.2.1.2 Spin-lattice relaxation time (T_1) variations. The investigated HT-BTF reaction occurs in aqueous solution with substantial pH changes (see Figure1.3) during the reaction. pH changes generally lead to a change in the longitudinal relaxation time T_1 of the water protons, and often also to a change in their chemical shift. Both T_1 and

chemical-shift changes can influence the success of a chemical-shift-selective pulse sequence such as EXCEPT. It is usually very time consuming to re-adjust the NMR parameters of a solvent suppression sequence if the relaxation time or the chemical shift of the solvent magnetization changes. EXCEPT was specifically developed to accommodate an order of magnitude variation in T_1 without the need for re-optimizing NMR parameters. It is one of the goals of this work to show that EXCEPT fulfills the theoretical predictions that lead to its design.

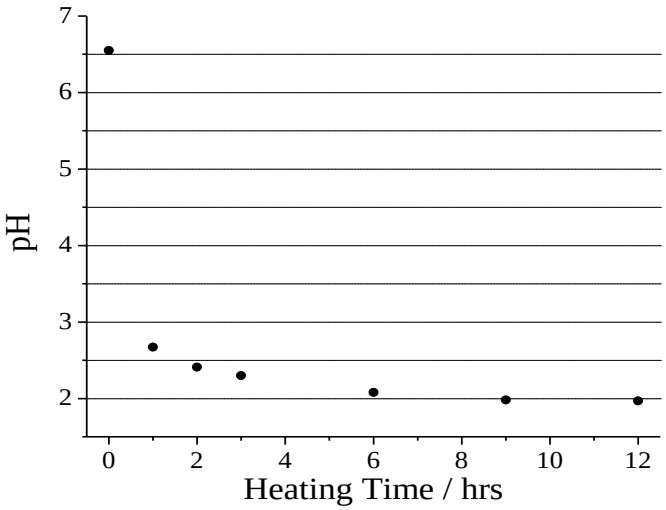

Figure 1.3. pH changes over the course of HT-BTF reaction

1.2.2. Integration Standard. In qNMR, integration standards are needed to compare signals from unknown concentrations to the signals of a known concentration.

Two basic techniques are used to insert an integration standard into an NMR tube for quantitative measurements. The first technique uses an internal reference, which consists of a known amount of a specific material that is added directly into the solution under investigation. In this research, maleic acid was chosen as internal reference, because it does not react with the other compounds in the solution, it exhibits only one singlet peak, and its signal falls in a chemical-shift range where no other analyte signal is found. However, the lock solvent (deuterated water, D_2O) for stabilizing the static magnetic field of the NMR magnet causes proton-deuterium (H-D) exchange between D_2O and maleic acid, which makes quantitative NMR measurements problematic. Work for this thesis therefore focused exclusively on the second technique which is the use of an external reference. . Using an external reference means adding a reference material of well-defined concentration in a capillary tube and then placing the capillary tube concentrically inside the NMR tube that contains the analytes. This way, the reference material does not come in contact with the analytes, and chemical reactions or cross contaminations can be avoided. To also avoid H-D exchange the solution containing the analytes does not contain any deuterated solvent.

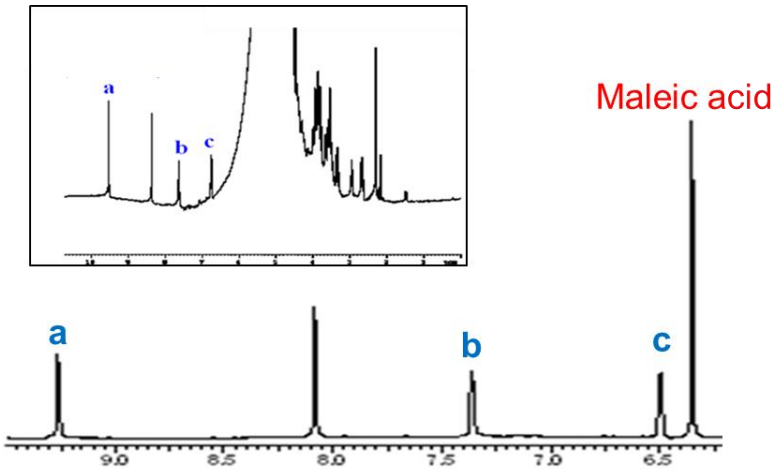

Figure 1.4. An expanded ^{1}H NMR spectrum of a 12 hours HT-BTF reaction solution, where an external reference (maleic acid) was used

1.3. OBJECTIVE

The main objective of this thesis is to introduce the development of Capillary-tube Package (CapPack) devices, which covers a new concept for testing the effectiveness of NMR pulse sequences. Specific objectives include:

- Describe the concept of CapPack devices as an invention to examine the effectiveness of NMR pulse sequences.
- Develop a microscale glass-sealing technique for manufacturing CapPack devices.
- Design an NMR-spectroscopic way to determine the volume factor in coaxial sample tube device.
- Use CapPacks and external references to show quantitatively the effectiveness of the newly developed NMR solvent-suppression pulse sequence called EXCEPT.

1.4. THESIS OVERVIEW

This thesis focuses on designing, manufacturing, characterizing and applying several newly developed NMR devices in applications of qNMR. The content of the thesis is arranged as follows:

Section 2 focuses on introducing the conventional methods for determining the distortions that solvent-suppression pulse sequences cause in NMR spectroscopy. In addition, the advantages of external references in qNMR measurements as well as the necessity to accurately determine the volume factor for external references will be discussed.

Section 3 focuses on experimental qNMR results obtained with different CapPack devices. To further enhance the robustness of CapPacks, a new microscale glass-sealing technique is introduced. Also, an NMR-spectroscopic method is employed to determine *in situ* the volume factor of coaxial sample tubes. Two CapPack devices are used to test and optimize the new solvent-suppression sequence EXCEPT.

The conclusions and future work are summarized in Section 4.

2. METHODOLOGY

2.1. SOLVENT SUPPRESSION CALIBRATION

Solvent suppression allows recording weak signals of solutes, which are otherwise distorted by overbearing solvent resonances. In aqueous solutions, the concentration of water protons is about 110 M, while solute concentrations are often 1 mM or less. In situations like this, it is necessary to suppress the NMR signal arising from the solvent. The water suppression scheme is one of the elements in NMR that most impacts the overall quality of a recorded spectrum. The choice of the solvent suppression scheme and of the associated parameters has therefore a high impact on the accuracy of the resulting spectra. As a consequence, potential users of ^{1}H NMR quantitative metabolomics would certainly benefit from a set of practical tools and recommendations to choose the experimental parameters leading to the most accurate and precise analysis of ^{1}H NMR spectra with solvent suppression.[14]

2.1.1. Irradiation Bandwidth. It is typical to test a solvent suppression sequence for its effectiveness by monitoring how successful it irradiates the entire solvent peak, which is usually the dominant peak in the NMR spectrum. The primary goal of the test is to determine the irradiation bandwidth (i.e., the range in the spectrum that is suppressed) from the suppression profile. Adjusting specific parameters of the solvent suppression sequence, such as the pulse profile and pulse length, is a common way to optimize the effectiveness of the applied sequence. There are two conventional methods for mapping solvent suppression profiles.

2.1.1.1 Z-field gradient irradiation. Figure 2.1.shows a z-field gradient test for observing a suppression profile: (a) a regular solvent signal (e.g., water), (b) the same

signal but with the application of a constant z-field gradient. The water peak has been evenly stretched out over a wide spectral range into a rectangular profile, (c) by applying a solvent-suppression pulse sequence at the center on the rectangular profile, a pulse-sequence-specific suppression profile is observed. The width at half-height of the curve presented in figure 2.1 (c) is termed the suppression bandwidth. An advantage of this technique is that the suppression profile is observed with a single NMR experimental; the disadvantage is that inaccuracies in the magnetic field gradient automatically translate into inaccuracies in the suppression profile.

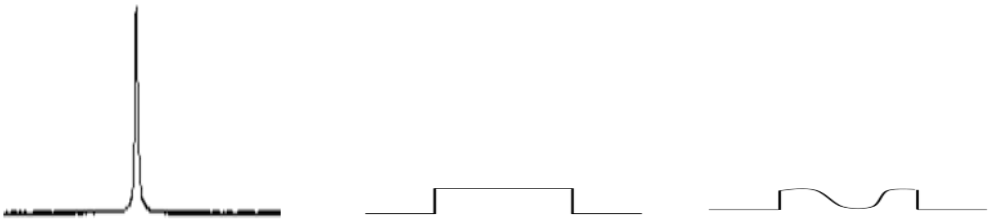

Figure 2.1. Application of a constant z field gradient to observe the suppression profile of a solvent-suppression pulse sequence

2.1.1.2 Selective irradiation with transmitter frequency offset. Figure 2.2 shows (a) a regular solvent signal (e.g., water). By applying a solvent-suppression sequence in multiple steps with small increments (e.g., 100 Hz) along the spectral range of interest (e.g., 12 steps starting at 600 Hz below and ending at 600 Hz above the solvent's resonance frequency, see Figure 2.2), the solvent-suppression profile is mapped.

The suppression effectiveness is now calculated from the integrated intensity of the undisturbed solvent signal over the intensity of the remaining solvent signal recorded with the suppression sequence. Equivalent to the z-gradient irradiation, the profile width at half height is called the suppression bandwidth. The advantage of this technique is that the selective irradiation frequency can be moved along the spectral range by very small amounts (a fraction of 1 Hz) in order to map the suppression profile very accurately. However, the number of points used to map the suppression profile can then be quite large. The finer a suppression profile is mapped the longer it will take to perform the procedure. In the example presented below (b in Figure 2.2), the rather coarse mapping of the suppression profile will already take 12 times as long as a single NMR experiment. Thus, the determination of a suppression profile can take a multiple of the amount of time needed to run the actual NMR experiment in which the suppression sequence is utilized

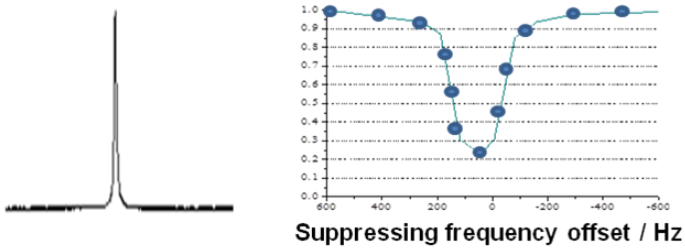

Figure 2.2. Stepwise measurement of a solvent-suppression profile

2.1.2. Spin-lattice Relaxation Time (T_1). Spin-lattice relaxation time (T_1) is the amount of time required for nuclei to recover longitudinal magnetization, and is described by the equation

$$M_z = M_0 [1 - 2 \exp (- t / T_1)] \quad (1)$$

where M_z is the current longitudinal magnetization and M_0 the magnetization at thermodynamic equilibrium. T_1 can be greatly affected by reaction conditions such as pH, concentration of paramagnetic ions, or temperature. One of the primary goals in testing the newly developed EXCEPT pulse sequence is to demonstrate its insensitivity to variation in the spin lattice relaxation time (T_1)

2.1.2.1 T_1 control tests. $CuSO_4$ in aqueous solution causes the local magnetic field in the solution to change slightly. In addition, it reduces dramatically the spin-lattice relaxation time of the solvent protons (i.e., water protons). To better understand how well a solvent suppression sequence is able to suppress samples of different spin-lattice relaxation times, a groups of six model samples in 5-mm NMR tubes was prepared. Each sample tube contained the same nominal concentration of water protons but a different amount of the nuclear spin relaxation agent $CuSO_4$ (Table 2.1). A set of standard NMR experiments with the six different 5-mm NMR samples, each with a different concentration of $CuSO_4$, revealed that the chemical shift of the water proton signals shifts to low field as the $CuSO_4$ concentration increases (Figure 2.3) and simultaneously the T_1 relaxation time of water proton gets shorter (Table 2.1 and Figure 2.4).

Table 2.1 Concentration of $CuSO_4$ solution

No.	a	b	c	d	e	f
$CuSO_4$ (M) (± 0.001 M)	1.000	0.750	0.500	0.250	0.125	0.062
T_1 (s) (± 0.00005 s)	0.01865	0.02289	0.04254	0.1089	0.1275	0.7574

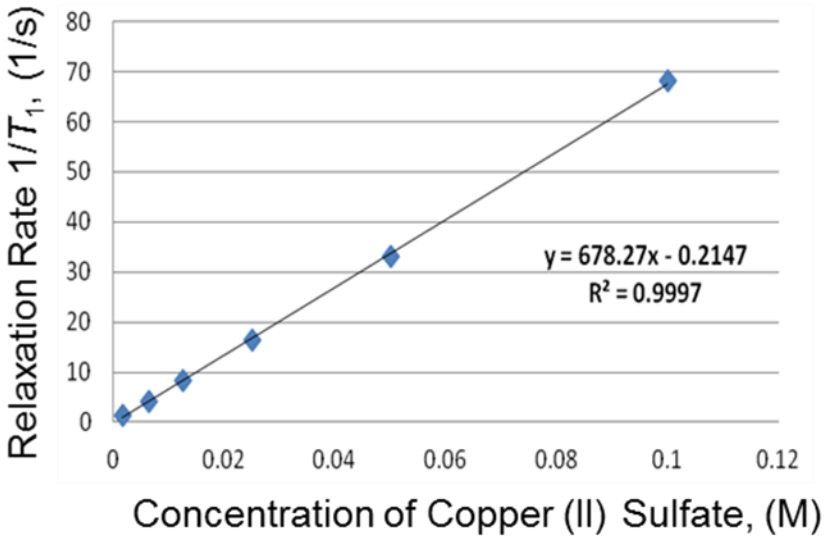

Figure 2.3. Concentration of $CuSO_4$ solution vs chemical shift of the water proton signal

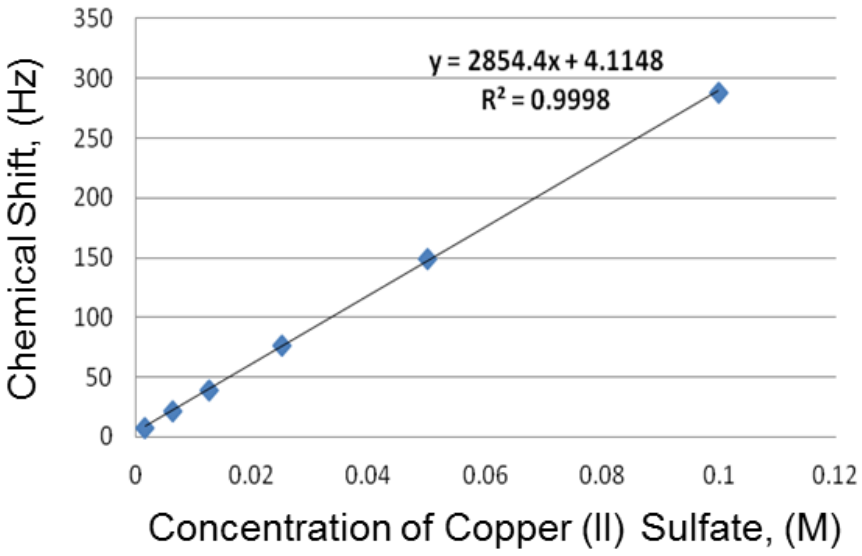

Figure 2.4. Concentration of $CuSO_4$ solution vs relaxation time of water proton signal

2.2. INTEGRATION CALIBRATION

Nuclear magnetic resonance (NMR) spectroscopy is an inherently insensitive analytical technique. However, NMR is an incredibly powerful technique. By increasing the magnetic field strength, the number of spins in the testing sample, and the number of individual scans for a measurement, NMR signals in the spectrum can be substantially enhanced. Accordingly, NMR can, for example, be used for measuring the purity of samples. qNMR (particularly ^{1}H qNMR) has become a routine technique in many academic and industrial settings, and it has produced a wealth of techniques and knowledge[15], e.g., in pharmaceutical and chemical analysis, or in the food industry. One plausible interpretation of this phenomenon is that proprietary qNMR methodology provides a competitive advantage for industrial companies. All of this indicates the

superiority of qNMR capabilities in terms of work-flow effectiveness, accuracy, precision, and cost−benefit relationships, when compared with other established methods. Still, all qNMR experiments require two forms of quantitative adjustment: one concerns the chemical shifts (δ in ppm) of the resonances, and the other relates to the integration of the NMR signals. For these calibrations, both internal and external references are used. Research described in this thesis is mainly focused on quantitation enhancement of ^{1}H qNMR with the use of external references.

2.2.1. Calibration Standard. In one-dimensional NMR experiments without hyperpolarization or polarization transfer from other nuclei, the integration over an NMR signal is directly related to the number of nuclear spins in the sample. Therefore, a known amount of reference material could be added directly into the solution or a reference solution with a well-known concentration could be placed in a separate tube within the NMR sample tube and hence used as external reference.

2.2.2. Coaxial sample-tube Device. In this work, a coaxial sample-tube arrangement consisting of a 1-mm capillary inside a 5-mm NMR sample tube was assembled to place the integration reference standard maleic acid together with the deuterium lock solvent (D_2O) in the center of the NMR sample tube. (Figure 2.5).

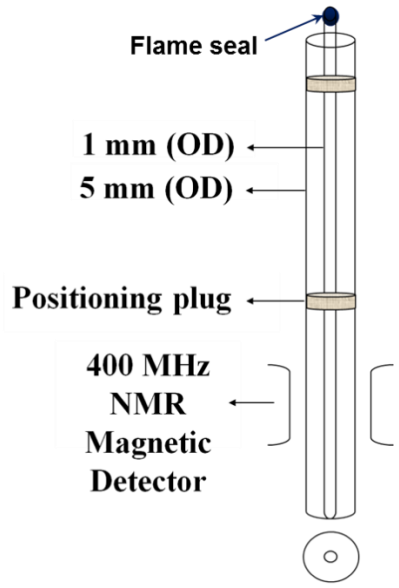

Figure 2.5. Coaxial sample-tube device

2.2.2.1 Volume factor. In science, engineering, industry, and statistics, the accuracy of a measurement is the degree of closeness of the measured quantity to that quantity's true value. In order to accomplish more accurate result, data acquisitions for quantitative NMR experiments must be collected under very specific conditions. In some aspects, the primary condition ensures that all analytes to be quantified and any added reference compound each have at least one completely resolved signal in the spectrum. In addition, the intensity for each of these signals must be an absolute representation of the total number of nuclei giving rise to the signal.[1] For an external reference setup as described above (Figure 2.5), it is important to determine the relationship of the sample volume to the reference volume (i.e., the volume factor) to the highest accuracy possible.

The volume factor is needed to calculate concentrations of the sample compounds from the known concentration and the integrated signal intensity of an external reference.

Table 2.2 shows the general methods to calculate the volume factor, while Table 2.3 shows the properties of NORELL NMR tubes used for the experiments in this thesis.

Table 2.2. Methods of calculating volume factor

Method	Procedure	Purpose
Geometric	Tube properties from manufacture	Standard and simple
Gravimetric	weight	Very accurate
NMR spectroscopic	NMR test	Most direct

Table 2.3. The properties of the coaxial sample-tube device

	I.D. / mm	O.D. / mm
NMR Tube	**4.2± 0.03**	4.97±0.03
Capillary Tube	0.8 ± 0.009	**1.0 ± 0.009**

By using the values marked in bold in the table of the coaxial sample tubes, the following equation is used to calculate the sample cross section:

$$S_{sample} = \pi[(\frac{d_{I.D.}}{2})^2 - r\frac{d_{O.D.}}{2})^2] \quad (2)$$

Similarly the I.D. of the capillary tube is used to calculate the cross section of the reference using the following equation:

$$S_{reference} = \pi[(\frac{d_{I.D.}}{2})^2] \quad (3)$$

According by, a geometric volume factor of $S_{sample} / S_{reference} = 26 \pm 1.87$ (7%) is determined.

3. EXPERIMENTS AND DEVICE

3.1. CAPPACK DEVICES

A CapPack device includes one or more capillary tubes arranged in geometries, including: concentric and side-by-side. A concentric tube model was used for an integration reference. A side–by-side tube model was used for performing suppression profile and T_1 variation experiments. In this thesis, the CapPack devices produce two unique NMR signal profiles that enable the determination of several important performance parameters for solvent-suppression sequences: dispersion, degree of suppression, and T_1 insensitivity. Two CapPack devices will be discussed: Gradient CapPack device and T_1 CapPack device.

3.1.1 Gradient CapPack Device. The gradient CapPack device consists of a series of up to nine capillary tubes (I.D.: 25 μm, O.D.: 325 μm) filled with the same aqueous $CuSO_4$ solution. The capillary tubes were inserted into a standard 5-mm NMR tube such that they were aligned parallel to the long axis of the 5-mm tube. An additional, flame sealed 1-mm NMR tube filled with maleic anhydride (0.50 M in acetone-d6) as an integration reference with some $Cr(acac)_3$ powder as a relaxation agent, was placed inside the 5-mm NMR tube. (Figure 3.1)

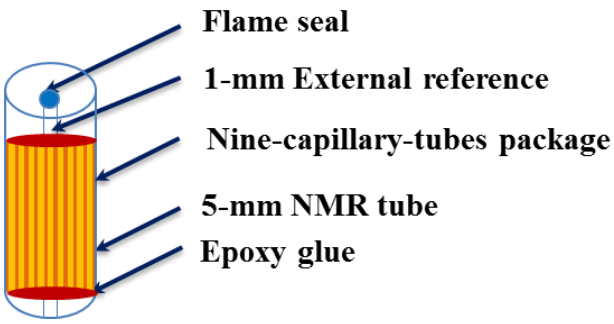

Figure 3.1 Gradient CapPack device for testing solvent-suppression irradiation profile

Figure 3.2 shows the ^{1}H NMR spectrum of a gradient CapPack device tested using a 400 MHz Varian liquid state NMR spectrometer. The water proton signal (Fig. 3.1) represents nine water proton signals, from nine capillary tubes in the gradient CapPack. Because the static magnetic field of NMR magnet is homogenous the nine sharp signals overlapped into one sharp signal. When a constant x and a constant y field gradient was applied across the set of capillary tubes, the nine water proton signal from the capillary tube solutions separate into nine peaks at regular intervals. (Figure 3.3)

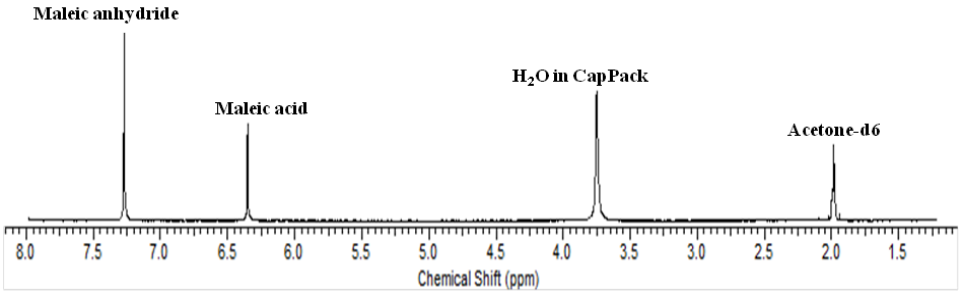

Figure 3.2. ^{1}H NMR spectrum of Gradient CapPack without the application of a constant x and a constant y field gradient

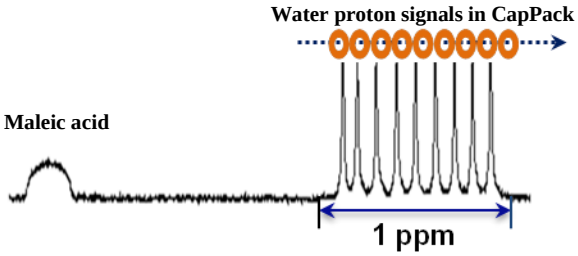

Figure 3.3. A zoomed in portion of the ^{1}H NMR spectrum shown in Fig. 3.2 with the application of a constant x and a constant y field gradient across the gradient CapPack

3.1.2. T_1 CapPack Device. The T_1 CapPack device consists of three components (see Figure 3.5): a set of six parallel capillary tubes (I.D.: 75 µm, O.D.: 364 µm, length: 6 cm); a 1-mm capillary tube; and a 5-mm NMR tube. The six capillary tubes were filled with a series of six different concentrations of aqueous $CuSO_4$ solutions (Table 2.1); the 1-mm capillary tube was filled with maleic anhydride solution (0.5 M in acetone-d6); and the 5-mm NMR tube contained the six parallel capillary tubes and the 1-mm capillary tube. The $CuSO_4$ solutions were chosen to cover an order of magnitude variation in T_1

(Table 2.2); the maleic anhydride solution was used as an integration reference and for locking; the 5-mm NMR tube was used as a container.

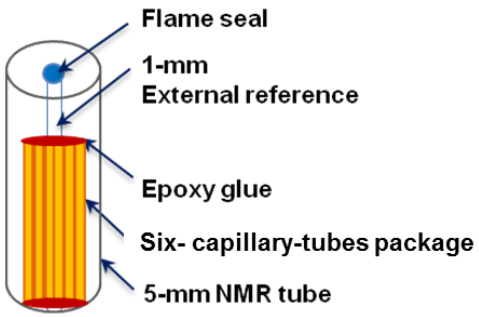

Figure 3.4. T_1 CapPack device for testing the effectiveness of solvent-suppression sequence for various T_1s

The T_1CapPack device was designed for testing variations in T_1 on the effectiveness of solvent-suppression pulse sequences, such as the EXCEPT solvent-suppression pulse sequence. A standard 90˚ pulse experiment using the T_1 CapPack device revealed six resolved peaks from the six capillary tubes (Figure 3.5). Each water proton peak represented a different concentration of the $CuSO_4$ solution, which correlated with the T_1 of water protons in that solution. When a constant x and a constant y field gradient was applied across the T_1 CapPack, the proton NMR signals from the six capillary tubes were made to merge into one broad signal (Figure 3.6).

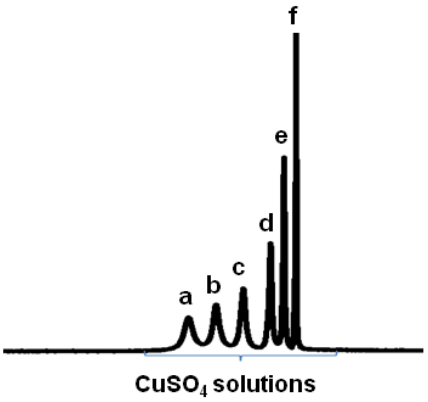

Figure 3.5. A zoomed in portion of ^{1}H NMR spectrum of water proton signals of the T_1 CapPack without the application of a constant x and constant y field gradient

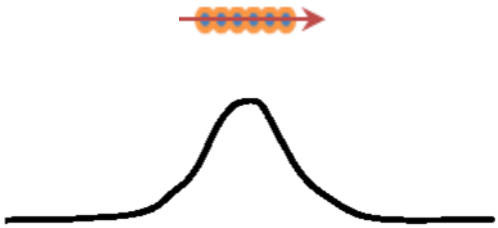

Figure 3.6. A zoomed in portion of the ^{1}H NMR spectrum with the application of a constant xy-field gradient across the T_1 CapPack

3.1.3 Glass-sealing Technique. In CapPack devices, sealing capillary tubes, which are filled with solutions, is important. Manufacturing tools for doing qNMR determinations, well-defined sample concentration and constant testing volume are the basic contents that need to be kept. Epoxy was used to hold the capillary tubes side by side. Also, it was used for temporarily sealing both ends of the capillary tubes. In general, it is a challenge to seal microscale capillary tubes (e.g., I.D.: 75 µm, O.D.: 364 µm) with

a flame. A novel technique to form a microscale glass-seal at both ends of the capillary tube was developed in this work. In addition, the sealed tube is able to survive at high temperatures and pressures in harsh environments. This technique involved an electric arc fusion approach utilizing an optical fiber arc fusion splicer: SUMITOMO Type-36 SM MM Core Alignment Fiber Fusion Splicer (Figure 3.7). The technique makes it possible to manufacture Gradient and T_1 CapPack devices, which can be used for qNMR calibrations of solvent-suppression pulse sequences. Moreover, those CapPack devices can serve prominently.

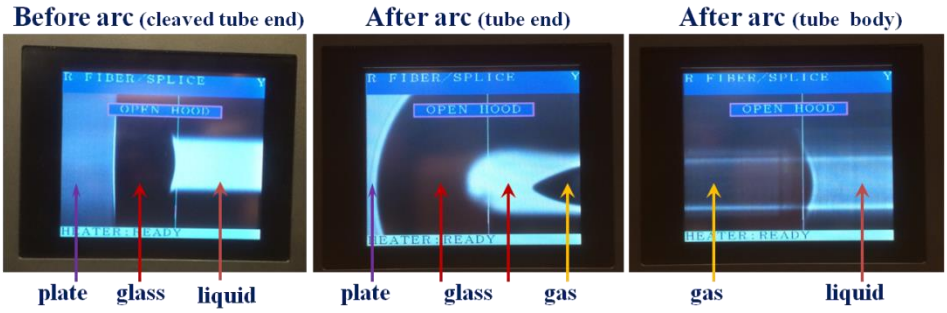

Figure 3.7. The images of microscale glass-sealing capillary tube in an arc fusion splicer

3.1.4 Applications of EXCEPT.

3.1.4.1. Gradient CapPack device. Figure 3.4 shows the overlapped nine water proton signals from the capillary tube solutions (Figure 3.3) split into nine NMR peaks at regular intervals. By applying the EXCEPT pulse sequence across the set of capillary

tubes, the water proton peaks from the capillary tube solutions were suppressed according to the selective radiation bandwidth profile. (Figure 3.8)

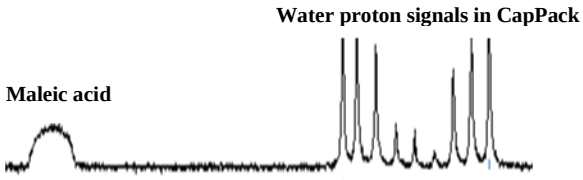

Figure 3.8. The ^{1}H NMR spectrum of the water proton signals with the application of the EXCEPT solvent-suppression pulse sequence at the center of the range of the water proton signals shown in Fig 3.3

By accurately integrating the nine water proton peaks before and after the application of the EXCEPT pulse sequence, the distortion factors can be calculated (Table 3.1).

Table 3.1. The distortion factors of the water proton signals after the application of the EXCEPT solvent-suppressing pulse sequence

Tube number	1	2	3	4	5	6	7	8	9
Distortion factor	0.00	0.00	0.15	0.68	0.70	0.85	0.22	0.08	0.00

3.1.4.2. T_1 CapPack device. Figure 3.6 shows the six water proton signals from the capillary tube solutions (Figure 3.5) overlapped into one broad NMR peak. By applying the EXCEPT solvent-suppression pulse sequence to the broad water proton signal, over 90% of the signal intensity was diminished. (Figure 3.9) Thus, the T_1 CapPack device incorporated six test samples with a range of T_1s, which made it less time-consuming in adjusting a standard set of parameters for the EXCEPT solvent-suppression pulse sequence. Therefore, it is much easier to maximize the water signal reductions of all six T_1s simultaneously.

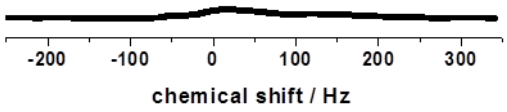

Figure 3.9. The ^{1}H NMR spectrum of the water proton signal after the application of the EXCEPT solvent-suppression sequence at the center of the broad water proton signal (Fig. 3.6).

Thus, the Gradient CapPack and T_1 CapPack devices are more time efficient for examining and optimizing the standard set of parameters for effective and successful solvent-suppression.

3.2. NMR SPECTROSCOPIC VOLUME FACTOR

A salient feature of ^{1}H qNMR is to determine the relationship of the sample volume to the reference volume (i.e., the volume factor) to the highest accuracy possible. Volume factor is needed to calculate concentrations of sample compounds from the known concentration and the integrated signal intensity of an external reference sample. Besides the geometric method to calculate the volume factor of coaxial sample tube devices, this thesis developed a new method, called NMR spectroscopic volume factor,

Electrons around the proton create a local magnetic field that opposes the static field. Since this induced magnetic field reduces the field experienced at the nucleus, the electrons effectively shield the proton. Thus, the frequency of the proton will change depending on the electron shielding around the proton. Paramagnetic copper ions in aqueous solution cause the local magnetic field in the solution to change slightly. This phenomenon was used to measure the volume factor of coaxial sample tube device. (Figure 3.10)

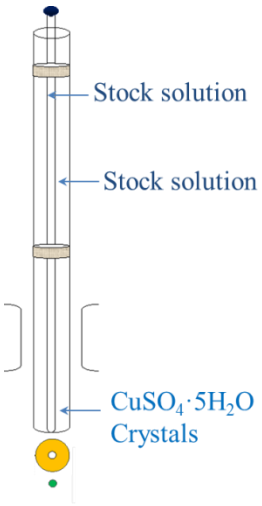

Figure 3.10. Co-axial tube device for NMR spectroscopic volume factor measurement

To calculate the volume factor of a coaxial sample tube device from an NMR experiments, several steps need to be followed. a) Fill the 5-mm and the concentric 1-mm NMR tubes with the same stock solution (1.00 M maleic anhydride in D_2O); b) apply a standard 90˚ pulse NMR experiment; c) carefully dissolve small, individual single crystals of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in the 5-mm-tube solution; d) apply a standard 90˚ pulse NMR experiment; e) add $CuSO_4 \cdot 5H_2O$ crystals until peak could easily be de-convoluted and integrated. (Figure 3.11)

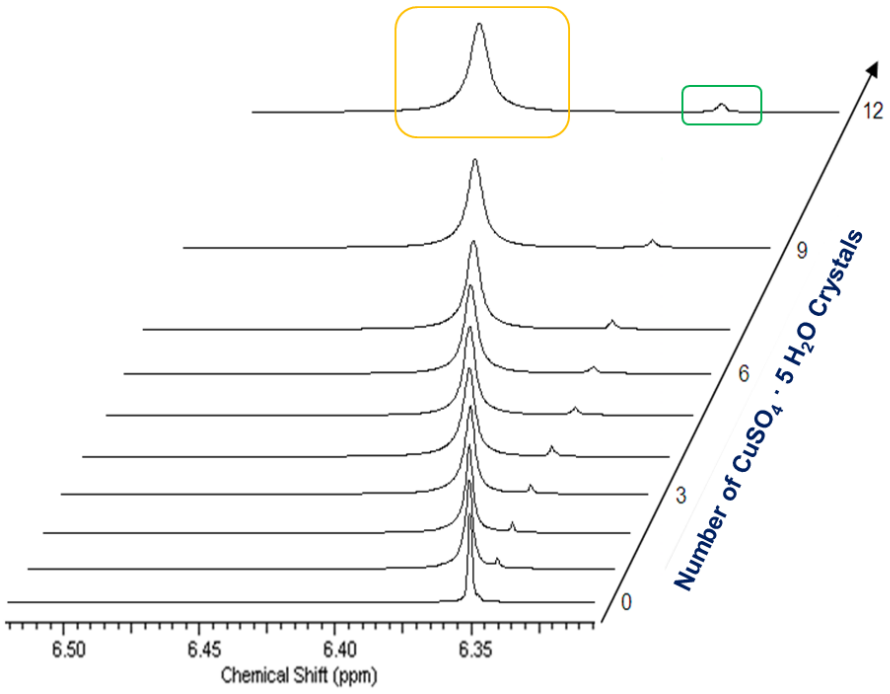

Figure 3.11. The static plot of ^{1}H NMR spectra as a function of increasing the number of $CuSO_4 \cdot 5H_2O$ crystals

In general, two ways were used to calculate the integration value. In one case, the regular way of doing integration cannot be right. The two peaks were overlapped or nearby each other, making it difficult in select the right range that could be used to calculate the integration values. In the second case, the deconvolution integration method coded in the Varian NMR Software (vnmr 6.1 B) was used. It modeled the peak with two separate equations, and processed the line fitting into two individual peak functions. Then, the method used the fitting functions to calculate the true integration values for the two signals. By using the deconvolution method, the NMR spectroscopic volume factor was calculated to be 27 ± 1.35 (5%). Figure 3.11 shows the deconvolution result of individual NMR signal ratios (blue dots) and the comparison between the NMR

spectroscopy volume factor (blue solid line) and the geometric volume factor (red dish line).

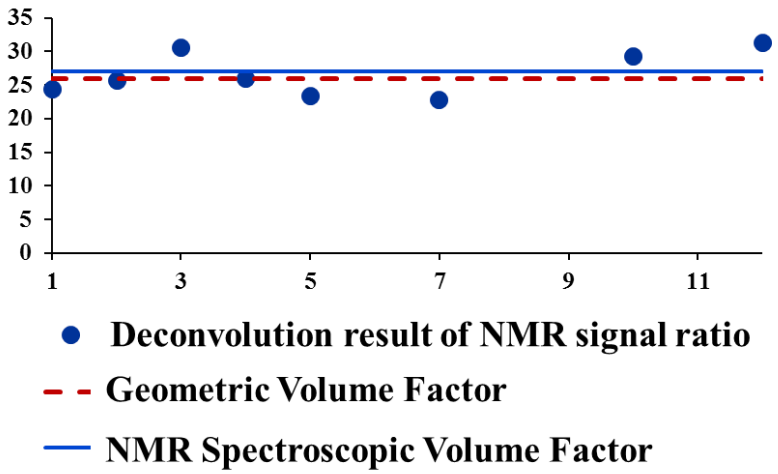

Figure 3.12. The comparison between geometric volume factor and NMR spectroscopic volume factor

4. CONCLUSIONS

4.1. CONCLUSIONS

For ^{1}H qNMR analysis of aqueous HT-BTF conversions, it was necessary to use an effective water-signal suppression sequence. Because strong variations of T_1 relaxation times occur during HT-BTF reactions, Dr. Woelk's research group developed the special solvent-suppression NMR pulse sequence EXCEPT. It is designed for suppressing solvent (water) signals reproducibly in samples drawn at different times during HT-BTF reactions. HT-BTF reactions typically last 12 hours but may also last up to 24 hours. During the reaction, the samples' T_1 times can change substantially. The Gradient CapPack device was developed to measure the suppression profile of EXCEPT, and to rapidly adjust its parameters and optimize the effectiveness of selective irradiation. The T_1 CapPack device was developed to adjust and optimize the standard parameters of EXCEPT to successfully cover an order of magnitude variation in T_1. Figure 4.1 represents NMR spectra of HT-BTF reaction products after 12 hours reaction at 150 °C before and after the application of the EXCEPT solvent suppression pulse sequence (upper and lower spectrum, respectively). The signals 1, 3 and 4 are assigned to 5-HMF, signal 2 is from formic acid, signal 5 from the external reference maleic acid, signal 6 and 7 are from citric acid, signals 7, 8, 9 from levulinic acid, and signal 10 is from lactic acid. With optimized parameters the EXCEPT pulse sequence effectively suppresses the water-proton signal at about 4.7 ppm, while the HT-BTF products can still be accurately quantified. With a coaxial sample tube device, and a well-defined NMR spectroscopic volume factor, the reaction products were determined for their absolute amounts.

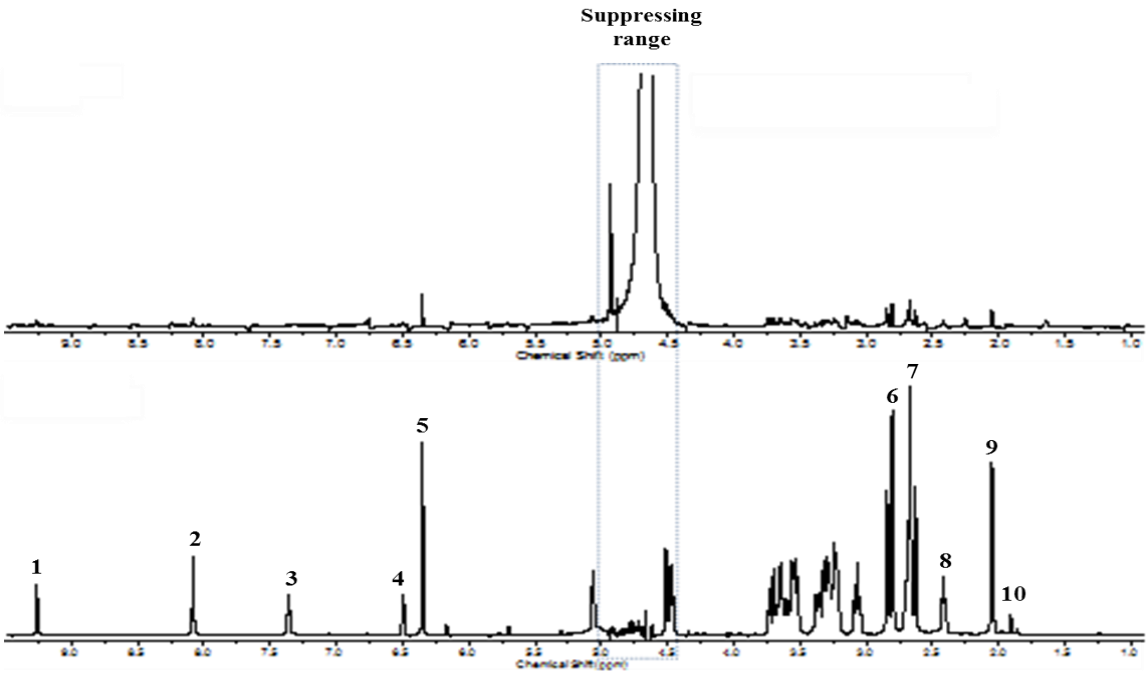

Figure 4.1. 400 MHz ^{1}H spectra before and after the application of EXCEPT solvent-suppression pulse sequence of HT-BTF products after 12 hours reaction, at 150 °C

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VITA

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