MEASUREMENT AND MINIMIZATION OF FIELD INHOMOGENEITIES IN HIGH RESOLUTION NMR

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Abstract

In this work, the homogeneity of both the $B_0$ and $B_1$ fields was studied. Both $B_0$ and $B_1$ field homogeneities are the basic assumptions of high resolution liquid state NMR. Although some inhomogeneity of both of the fields is always present, the spectrometers can be operated, with the help of the developed spectral purging techniques, without giving any thought to the field inhomogeneities or the necessary actions to minimize their adverse effects. Although the effect of $B_0$ inhomogeneity can occasionally be seen, the $B_1$ field in a modern probe head is often assumed to be sufficiently homogenous for any practical purpose. By using the method used in this study the $B_1$ field strength along one axis, typically the z-axis, can be easily mapped. Based on the information gathered from a single experiment, one can obtain reliable and valuable information about the $B_1$ field distribution, e.g. homogeneity of the coil. From such information, the degree of required artifact suppressing methods for successful NMR experiments can be determined. Since normal pulse length calibration also requires the acquisition of several 1-D spectra, the required experimentation time is not increased.

Although the maximum amount of signal from an NMR experiment is obtained when the signal is acquired from a maximum number of resonating spins, the results presented show that significantly more homogenous $B_1$ field along the active sample volume is achieved by rejection of the signal originating from the outer parts of the coil length. Although the total amount of signal obtained from the outer parts of the RF-coil is not very high, some loss of signal is associated with the spatially selective acquisition. The rejected signal, however, is a significant source of artifacts, and if no precautions were taken, the artifacts would severely decrease the quality of the acquired data. If the sample concentration can be increased, it would be advantageous to dissolve the amount of sample available in as small an amount of solvent as is possible and place the sample in the most $B_1$ homogenous part of the probe-head RF-coil. With the same amount of nuclear spins concentrated into a smaller volume, the sensitivity of an NMR experiment can be increased manifold.

As an application of a spatially selective data acquisition, a versatile method capable of producing a map of the $B_0$ field strength and its variation along the sample volume is presented.

Keywords: NMR, homogeneity, sensitivity, localization
"Ei riitä että on paras. Täytyy olla ylivoimainen."

–Antti Kasvio
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## Symbols and abbreviations

- \( B_0 \): Static magnetic field
- \( B_1 \): External magnetic field
- BBI: Broad Band tunable X-channel equipped probe head optimized to Inverse i.e. proton detection
- BURP: Band selective, Uniform Response, Pure phase
- COSY: COrrelation SpectroscoPY
- DEPT: Distortionless Enhancement through Polarization Transfer
- EB: Ethyl Benzene
- FID: Free Induction Decay
- FT: Fourier Transform
- HSQC: Heteronuclear Single Quantum Coherence
- INEPT: Insensitive Nuclei Enhancement by Polarization Transfer
- ISIS: Image-Selected In-Vivo
- M: Magnetization
- MRI: Magnetic Resonance Imaging
- NMR: Nuclear Magnetic Resonance
- MRS: Magnetic Resonance Spectroscopy
- NOE: Nuclear Overhauser Enhancement
- NOESY: NOE SpectroscoPY
- PFG: Pulsed Field Gradient
- PRESS: Point-RESolved Spectroscopy
- RF: Radiofrequency
- STEAM: STimulated Echo Acquisition Mode Spectroscopy
- TOCSY: Total COrrelation SpectroscoPY
- TXI: Triple channel probe head with additional X-nucleus, optimized to Inverse (i.e. proton) detection.
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1 Introduction

1.1 Magnetic resonance

The fundamental property of the atomic nucleus is the nuclear spin \( I \), which has values of 0, \( \frac{1}{2} \), 1, \( \frac{3}{2} \), etc, in units of \( \hbar/2\pi \). The nuclear magnetic moment \( \mu \) is directly proportional to the spin \( I \), i.e.

\[
\mu = \frac{\gamma I \hbar}{2\pi},
\]

where \( \gamma \), the proportionally constant, is called the magnetogyric ratio and is a constant for each particular nucleus. A nucleus of spin \( I \) has \( 2I + 1 \) possible orientations, which are given by the value of the magnetic quantum number \( m_I \), which has values of \( -I, -I + 1, \ldots, I - 1, I \); i.e. for a nucleus of spin \( \frac{1}{2} \), \( m_I \) has values \( \pm \frac{1}{2} \). Examples of the spin \( \frac{1}{2} \) nuclei are \(^1\text{H}, \ ^{13}\text{C} \) and \(^{15}\text{N} \).

The energy of interaction is simply proportional to the nuclear moment and the applied field. By using Eq. (1.1) one gets

\[
E = -\frac{\gamma I \hbar}{2\pi} m_I B_0,
\]

where \( B_0 \) is the main magnetic field [1, 2, 3].

The spin can flip spontaneously from one orientation (energy state) to the other as the spin is in a large magnetic field. Such a flip is a relatively infrequent event spontaneously, but if energy that is equal to the differences in energy (\( \Delta E \)) of the two nuclear spin orientations is applied more flipping will occur. The irradiation energy is in the RF range. The absorption of energy by the nuclear spins causes transitions from higher to lower energy levels as well as from the lower to the higher energy level. The energy absorbed by the spins induces a voltage that can be detected by a suitably tuned coil. This voltage, after being amplified, can be displayed as a free induction decay (FID).

The energy required to induce spin flip and cause an NMR signal appearance is just the energy difference between the two nuclear orientations, and is dependent on the strength of the magnetic field \( B_0 \) (See Fig. 1) [1, 2, 3].
\[ \Delta E = \frac{\gamma h B_0}{2\pi}. \]  

(1.3)

Fig. 1. Energy levels and transitions for a nucleus \((I = \frac{1}{2})\) in a magnetic field \(B\).

1.1.1 Boltzmann distribution

The whole phenomenon of magnetic resonance depends on the difference in population between the two energy states, \(\alpha\) and \(\beta\). The Boltzmann equation

\[ \frac{N_\beta}{N_\alpha} = e^{-\frac{\Delta E}{kT}} = e^{-\frac{h\nu}{kT}}, \]  

(1.4)

where \(k\) is the Boltzmann constant, \(T\) is the absolute temperature. From the equation one can calculate that at room temperature in an 11.4 T magnetic field, which corresponds to the 500 MHz proton frequency, the ratio has a value of 0.999919998, which corresponds to the situation where we would have, for every million spins, an excess of 40 spins in the lower energy state.
1.1.2 Magnetic susceptibility

The response of the internal magnetization of a material to an applied external magnetic field is referred to as its magnetic susceptibility. Both orbital and spin angular momenta of electrons generate a magnetic field \( B \). The magnetic moment of a single particle is inversely proportional to its mass. Therefore, the nucleus contributes much less to the magnetic properties of the atom than do the electrons. The electronic magnetic moment is about 650 times larger than that of the proton. Thus, nuclear magnetization is responsible for NMR signal, but electron magnetization determines the bulk magnetic properties of matter.

Most organic and many inorganic compounds are diamagnetic and have paired electrons. These materials reduce the magnitude of the external field by induction of an opposing field. No magnetic field arises from the spin angular momentum of paired electrons. Unpaired electrons, however, have both net spin and orbital angular momentum. Placed in the external magnetic field, electron magnetic dipoles align parallel or antiparallel to the applied field, thus producing a net magnetization. More dipoles align parallel to the field, enhance the external field, and this effect dominates the weaker diamagnetic properties, when sufficient unpaired spins. Materials that enhance the magnitude of the external field are said to be paramagnetic.

The induced magnetic field is proportional to the strength of the applied magnetic field, \( B_0 \):

\[
M = \chi B_0, \tag{1.5}
\]

where \( M \) is the induced magnetization. The constant of proportionality \( \chi \) is positive for paramagnetic materials and negative for diamagnetic materials [4].

Magnetic susceptibility is a measure of how much a matter affects a magnetic field around it. The higher the magnetic susceptibility the greater the effect the matter has on the magnetic field. Although magnetic susceptibility measurements can also be done by NMR, in normal spectroscopy one does not see direct susceptibility caused effects.

To avoid susceptibility incontinuities caused by sample boundaries, the sample height in the NMR experiment should be at least 3 to 5 times the height of the receiver coil. These incontinuities would introduce severe distortions in the \( B_0 \) field along the active sample volume. Should such \( B_0 \) field distortions be present in the sample, the effect would be severely broadened lines, a total lack of resolution and diminished signal-to-noise ratio. These distortions in the \( B_0 \) field strength are usually uncorrectable by the shim coil current adjustments [5, 6, 7]. For optimal signal acquisition, a homogenous \( B_0 \) and \( B_1 \) field should be present. Deviation in either field strength along the sample volume results in a spectrum with less than optimal signal width and intensity. Inhomogeneities in the \( B_0 \) field cause broadening of the signal, and if an NMR-method is based on the use of several pulses with distinct nutation angles, the amount of acquired signal diminishes in inhomogenous \( B_1 \) field. If there were no susceptibility concerns or limitations on increasing sample concentration, the optimal method for obtaining good quality NMR spectra would be to collect the signal from a very small sample volume. For
a small sample volume, the achievement of a homogenous $B_0$ field is a simpler task, and the variations in the $B_1$ field strength would also be smaller. The susceptibility incontinuity effect cannot be ignored, however, and the advantages of the aforementioned benefits can only be utilized if the perturbations to the $B_0$ field can be minimized [1, 2].

1.2 $B_1$ field

The $B_1$ field is the RF field generated by RF-amplifiers and RF-coils surrounding the sample in the magnet. The $B_1$ field is perpendicular to the static magnetic field. The purpose of this field is to manipulate the magnetization of the spins in the sample. As the $B_1$ field is applied at resonance frequency, normally in form of short, a few micro second long pulses, this causes the magnetization vector of spins at the resonance frequency to tip about the axis (in rotating frame) that pulse was applied.

For magnetogyric ratio $\gamma$, a pulse applied at the field strength $B_1$, and for duration $\tau$, a flip angle $\beta$ is obtained:

$$\beta = -\gamma B_1 \tau.$$  \hspace{1cm} (1.6)

1.2.1 RF-coil

In a modern, high field, high resolution NMR spectrometer probe head there are usually only two RF coils present. The first, doubly tuned to $^1H$ and $^2H$, is used for transmitting and receiving the deuterium lock signal, pulsing and receiving the proton pulses, and receiving the proton FID. The other coil is used for the $X$ channel, and if it is only single tuned, is normally used for $^{13}C$ detection and pulsing. Sometimes the other channel can be tunable to various $X$ nuclei. It is also possible that the second RF coil in the probe is double tuned to $^{13}C$ and $^{15}N$, and if such an arrangement is done, this coil is used to transmit the pulses on carbon and nitrogen channels, and should there be need, also to receive the FID signal. The latter configuration, with the double tunable second RF coil is nowadays widely used in biopolymer NMR research, where doubly $^{13}C$ and $^{15}N$ labeled samples are used as a norm. In any of the aforementioned probe head configurations the signal, or pulse, is transmitted and later the emitted signal from the sample is collected with the very same coil.

One of the parameters characterizing the coil is the quality factor $Q$. In general, the higher the $Q$, the greater the sensitivity, and the larger the RF field than can be obtained with a given RF power. $Q$ characterizes also the damping of the coil i.e. the lag time for any changes in the RF power or phase. The lag time in nanoseconds is approximately equal to the value of $Q$. Typical $Q$ values lie in the range from 300 to 400 [9, 10, 11]. The requirements for optimal receiver coil
and optimal transmitter coil can be very opposite. In addition, the optimal coil
dimensions for receiver and transmitter coils are not necessarily the same.

1.2.2 \( B_1 \) field homogeneity

Modern high resolution NMR methods rely on the capability of the spectrometer
to produce reasonably exact and uniform pulses. Real spectrometers are naturally
incapable of producing perfect RF pulses, but it is often believed that the contribu-
tion of \( B_1 \) inhomogeneity is so small that it may be mostly neglected. So, with the
aid of purging schemes such as phase cycling and gradient purging, the existence
of a relatively homogeneous \( B_1 \) field can be assumed. In some cases the occurring
\( B_1 \) field inhomogeneity has been utilized [12, 13, 14], and occasionally the effect
of \( B_1 \) field inhomogeneity has been taken into account [15, 16, 17], but most often
a homogeneous \( B_1 \) field is assumed. At least a moderate \( B_1 \) field homogeneity
is very crucial for the reasonable performance of the spectrometer. Furthermore,
some experiments that are most sensitive to the \( B_1 \) field homogeneity cannot be
adequately performed, if a sufficiently homogenous \( B_1 \) field is not available, at
least not without severe signal loss [18, 19, 20]. Overall, very precise calibration
of the pulse length is required, especially with those multi-pulse experiments in
which suppression of intense lines, typically water, is needed [21, 22, 23].

The standard method for measurement of probe head RF-field homogeneity is
to compare the signal intensity produced by a 810° pulse to that produced by a 90°
pulse. If the \( B_1 \) field homogeneity were ideal the only difference between the signal
intensities would arise from the relaxation loss during the longer pulse duration
required for causing the 720° longer mutation. With a standard high resolution
NMR spectrometer RF-field strengths of more than 25 kHz and values up to 50
kHz are no exception. The 90° pulse length is 5 to 10 \( \mu \)s, and the pulse length
increase required to achieve the 810° rotation is 80 to 160 \( \mu \)s. In such a short time
the effect of the relaxation can safely be ignored. Therefore, the change in signal
intensity is caused by the \( B_1 \) inhomogeneities along the sample volume, which
cause less than nominal magnetization rotation. As the collected magnetization is
not completely coherent along a certain position, the collectable net magnetization
is smaller, yielding a signal with smaller intensity [8].

At the moment, the most widely used method for measuring the homogeneity
is the comparison of the intensity of a signal generated by a 810° pulse with that
generated by a 90° pulse. This is achieved normally by running a series of 1-D
experiments in which the pulse length is constantly increased by each experiment.
The intensities for both 90° and 810° pulses are taken to be the highest values of
the peaks in the first and the third maximum in phase sensitive presentation (first
and fifth in absolute value presentation) [24]. In addition to comparing the signal
intensities produced by 810° and 90° pulses, similar information can be obtained
by comparing the signal intensities produced by a 450° and 90° pulses. The signal
intensity reducing effect of \( B_1 \) inhomogeneity is naturally less profound when 450°
pulse intensity is used for comparison (See Fig 2). The method described above
Fig. 2. The result of the $B_1$ homogeneity experiment. Pulse length is incremented by 1 µs steps.

also shows a $360^\circ$ pulse length with reasonable accuracy, from which calculation of the RF-field strength is straightforward. The most serious shortcoming of this method is that it only gives an estimate of the width $\Delta B_1$ of the distribution of the RF field. The information obtained is qualitative in nature. No information on how the inhomogeneities are distributed can be gathered. Generally, the intensity ratio values exceeding 70% are considered good for a modern high-resolution NMR probe-head. Although several methods for determining probe head $B_1$ homogeneity have been proposed [25, 26, 27, 28], one of the most elegant of those, a method that provides actual vision of the $B_1$ field distribution, was proposed by Jerschow and Bodenhausen [29]. The method relies on mapping the spatial dependence $B_1(x, y, z)$ by calculating the

$$\Theta(r) = \arccos \frac{f_{\text{STE}}(r)}{f_{\text{SE}}(r)}$$

of two three-dimensional imaging experiments, namely spin echo (SE) and stimulated echo (STE) experiments. The image representing the spatial distribution of the $B_1$ field can be mapped and a clear view of the probe-head $B_1$ field distribution can be obtained. However, according to the reference [29], the data acquisition takes nearly a full day, 23 h. Since no numerical data for the $B_1$ field values are represented, apparently no such information may be revealed from the acquired datasets.

The information about the $B_1$ homogeneity can also be approximated by acquiring a one or multi-dimensional image of a sample that is longer than the RF-coil. By measuring the image after small nutation angle pulse the resulting image is representative of the $B_1$ field strength along the axis in which the $B_0$ gradient
field is applied. As the intensity of the image varies relative to the both transmitter and receiver coil, which in a standard probe-head are the same coil, the variation in the $B_1$ field strength is shown squared. If the image is measured by using a pulse width corresponding nominally to the 180° magnetization rotation as the excitation pulse, no signal is observed from the regions where the actual magnetization rotation coincides with the nominal amount of rotation, but a visible signal is obtained if the RF-field strength is non uniform in some regions of the active sample volume [31].

1.3 $B_0$ field

The performance and, to a significant degree, the sensitivity of a high resolution NMR experiment is dictated by the $B_0$ field homogeneity. Excellent homogeneity can be achieved with a careful adjustment of the currents of the various shim coils of the modern high resolution NMR spectrometer [33].

1.3.1 Effect of shim-coils

The purpose of the shim coils is to adjust the $B_0$ magnetic field to be as equal along the sample volume as possible. Current applied to the shim coils causes magnetic field, and the coils are designed to have the spatial dependence of the magnetic field adjustment. The adjustment of the $z^1$ shim causes magnetic field changes linearly about the z (vertical) axis. Adjustment of the $z^2$ shim causes magnetic field changes along the z-axis that depend to the power of two on the distance to the center of the sample volume etc.

1.3.2 Manual homogeneity adjustment

1.3.2.1 Homogeneity adjustment by monitoring lock signal level

The most common method for adjusting the homogeneity of the $B_0$ field is to adjust the shim currents based on the level of the lock signal. With increasing $B_0$ homogeneity field is, the more spins of the deuterated solvent are resonating at the exact same resonance frequency, coherently adding to each other and increasing the amount collectable signal. When the magnetic field along the sample is not equal the spins at different parts of the sample do not resonate at exactly the same frequency and thus the lock signal level is lower. As the shim coil currents are being adjusted, the lock signal level is indicative of the total amount of spins resonating at the nominal magnetic field. It does not, however, give any indication of how
the magnetic field deviates. No information about the sign, amount or position of 
the area where magnetic field is non-equal to the nominal is gathered.

As only the lowest shim coil, \( z^1 \), can be adjusted to its optimal value by one 
dimensional optimization of the lock signal level, all the higher shim coils must 
be adjusted by n dimensional searches. Simply a task non trivial to perform and 
due its difficulty and the time requirements it takes to do it adequately various 
shortcuts are usually taken. Some of those can still result, depending on the 
original deviation conditions of the \( B_0 \) field from optimal, quite usable results. 
Some others can, however produce less than optimal results. Although the re-
sulting spectrum may show symmetric and reasonably narrow signals, the amount 
of signal obtained can be severely minimized compared to the optimal situation. 
Achieving an excellent \( B_0 \) field homogeneity by adjusting the shim coil currents 
based on the lock signal level, is still possible. It requires quite a lot of patience, 
however as well as time and skill.

1.3.2.2 Homogeneity adjustment by monitoring size and shape of FID

Another way of monitoring the quality of \( B_0 \) homogeneity while adjusting the shim 
coil currents is to record proton FID and, by the shape and intensity of it, adjust 
the various shim coils. The aim in this method is to obtain a FID signal that is 
decaying in single exponentially, and also to improve the area of the FID signal to 
the maximum. The integrated value of FID area can used as an indication of the 
total amount of spins resonating at the nominal frequency. This integration value 
contains in essence exactly the same information as the lock signal level does. It 
depends on current sample conditions as to which of the methods is more usable 
[43, 44].

1.3.3 Automated homogeneity adjustment

The manual adjustment of all the individual shims to their optimal value is very 
tedious work, and one usually settles for a less than optimal value, or the true 
optimum cannot be found in the time available, and the possibility to fall in to some 
local optimum in the course of the optimization process is substantial. Various 
techniques have been developed in order to assist in the course of shimming, such as 
the use of a simplex optimization routine, which is based on the observed intensity 
of the lock signal [34].

The most practical group of automated shim procedures takes advantage of 
the pulsed field gradient based imaging techniques. Normally, a shim map is 
acquired for each shim and the optimal shim settings can be calculated easily from 
these acquired maps. Correct shim settings, however, require that the current field 
inhomogeneity \( \Delta B_0 \) is smaller than it was during the acquisition of the shim maps. 
On the other hand, for the most accurate results, the inhomogeneity caused by the
shim setting should be of the same magnitude as are those to be corrected, based on the acquired field maps. Unless a variety of acquired field maps can be stored separately, one must either optimize the field map acquisition for achievement of the best possible shim settings, or for the possibility of being able to handle also a larger-than-normal shim miss-set. The method, however, is usable only in samples where a dominant proton signal is available, so this usually restricts the use of the method to samples dissolved in H$_2$O [35, 36, 37, 38, 39, 40, 41]. There is, however, the possibility of using a deuterium signal in a similar way as the proton signal, thus enabling the use of this method for samples dissolved in organic deuterated samples too. Unfortunately, the relaxation properties of the deuterium nucleus are not favorable to collecting the signal resulting in severe sensitivity loss. This loss is even more pronounced as the echo time should be longer proportionally to the magnetogyratic ratio $\gamma$ of the observed nuclei, resulting in increased pulse sequence duration. Further, because the sensitivity of an NMR experiment is proportional to $\gamma^{5/2}$ there is also sensitivity loss by a factor 108 for deuterium compared to protons. [42].

1.4 Spectroscopy

As spins are excited by radio frequency pulses, a simple one dimensional NMR experiment has performed. From the resulting spectrum, based on the the chemical shifts of the resonance signals and the scalar couplings various, valuable pieces of information can be obtained [45, 46, 47, 48]. This information can be used to great benefit in various studies in chemistry, biochemistry, physics, medicine and so on. In addition to the simple one pulse experiment described above multitude of various other more complex experiments can performed [49, 50, 51, 52, 53, 54, 55].

1.4.1 Multidimensional Spectroscopy

A large number of different kinds of multi-pulse experiments of varying numbers of dimensions have been published since the invention of FT-NMR [56, 57, 58]. Although the purpose, complexity and reason for various experiments varies greatly in each of them, manipulation of the spin vector orientation should preferably be controlled exactly by the spectrometer hardware as well as with the theoretical calculations [59, 60, 61, 62, 63, 64, 65] and simulations [66, 67, 68, 69, 70, 71, 72, 73, 74]. However, even the most complex multidimensional experiments are made up of relatively simple building blocks, like those used in COSY like experiments [75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85] or similar where magnetization transfer is based on Hartman-Hahn mixing [86, 87, 88]. The third important class of homonuclear experiments that is frequently used as a building block in more complex sequences are those based on the NOE, namely NOESY experiment [89, 90, 91, 92, 93, 94]. For the heteronuclear magnetization transfer the building
blocks used are usually the INEPT [95, 96, 97, 98, 99] based experiment together (to a lesser extent) with DEPT [100, 101, 102, 103] or cross-polarization based experiments [104, 105, 106, 107, 108, 109, 110].

1.4.2 Artifacts

With an ideal NMR spectrometer the resulting spectrum would look as predicted, and it would be only a matter of the accuracy of the prediction as to how closely the predicted spectrum would represent the actual spectrum [59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74]. However, an ideal spectrometer is hardly obtainable and several sources of artifacts are introduced to the spectrum. In order to obtain a \( \sqrt{2} \) gain in sensitivity the receiver is usually placed into the center of the spectral range. To be able to distinguish between positive and negative frequencies relative to the receiver a quadrature detection [111, 112] where two signal that differ in phase by 90° is collected (Fig. 3). Due to the slight differences between the two channels a image peaks may be introduced to the spectrum. By repeating the acquisition with different phases, the quadrature detection artifacts can be essentially eliminated [113, 114, 115, 116, 117]. In multidimensional spectra, the quadrature detection should also be arranged along the indirectly detected dimensions. This quadrature detection is usually arranged by acquisition of a parallel data set or by the time-proportional-phase-increment (TPPI) method [118, 119, 120, 121, 122].

In addition to the quadrature detection, other artifacts can also be suppressed by the phase cycle [50, 123, 124]. In some cases especially with multiple quantum coherence [125, 126], the understanding of the mechanism for phase cycling requires deeper analysis [127, 128, 129, 130, 131]. In addition to the phase cycling the desired coherence pathway [31, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141] can also be selected by pulsed field gradients [142, 143, 144], which also very effectively reduce the amount of spectral artifacts.

1.4.3 Selective excitation

The normal hard pulses optimally generate mutation that is equal over the whole spectral range. Based on the Heisenberg’s uncertainty principle, the shorter the pulse the wider range its response affects. For normal high power pulses, the mutating frequency range is roughly inverse to the length of the pulse. For an excitation that would only affect a certain part of the resonances and not affect the others, a normal, rectangle shaped, low power, long duration pulse is non-optimal due to the numerous intensive side lobes in its excitation profile.

Modification the amplitude of a pulse enables one to alter its excitation profile. The shape of a pulse and its excitation profile are not related by Fourier transformation [1]. More elaborate procedures must be used to find the optimal pulse...
Fig. 3. Schematic representation of one possible quad detection configuration in NMR spectrometer.

shape based on a desired excitation profile. A large selection of pulse shapes has been developed and characterized [145].

In general, a good amplitude-modulated pulse should have an adequate frequency selectivity, uniform excitation, uniform phase behavior and a short duration. Yet some of these desired properties contradict each other.

Amplitude-modulated pulses can be rather sensitive to the RF inhomogeneity of the coil in the probe and therefore require good RF homogeneity for best performance. The performance of an amplitude-modulated pulse depends on the initial state of the magnetization. A selective 180° pulse that provides good inversion properties for longitudinal magnetization in general, does not perform well as a 180° transverse magnetization. For this reason, some shapes of pulses, for example the BURP pulses, are grouped into families with a member for excitation, inversion and refocusing [146].

The most frequently used shaped pulses are Gaussian, sinc with either no or one pair of side lobes, Gaussian cascades, which are based on individual Gaussian-shaped pulses, and pulses of the BURP family [146, 147].

The BURP family of shaped pulses is quite a robust choice for selective pulses. Their performance is still rather good, the excitation outside of excitation bandwidth is minimal and the phase properties of excited pulses are excellent.

The center of excitation of a pulse can be changed also by changing its phase
during the application. The phase can be changed by a fixed frequency for an off-resonance shifted pulse or the frequency can be swept during a pulse. A pulse with fixed off-resonance frequency shift $\nu_{\text{off}}$ during the application can be obtained by linearly increasing the pulse phase $\Phi(t)$ with time while keeping the carrier frequency fixed at $\nu_0$:

$$\cos(2\pi\nu_0 t + \Phi(t)) = \cos(2\pi\nu_0 t + (\Phi_0 + 2\pi\nu_{\text{off}} t))$$

(1.8)

The increment per unit time depends on the required offset frequency $\nu_{\text{off}}$ from the carrier frequency $\nu_0$ [9].

When the phase $\Phi(t)$ in Eq. 1.8 depends nonlinearly on the time $t$, the effective frequency changes during the pulse. An important group of pulses using frequency sweeps during their application are the adiabatic pulse. These pulses excite, invert, or refocus magnetization over a very wide frequency range at the cost of a longer pulse duration and a phase dispersion across the excitation bandwidth. In applications where such pulses excite or invert magnetization, they are robust to RF inhomogeneities, but not when applied for refocusing [148].

Spectrometers software packages include required routines (Bloch simulator) which help to choose the appropriate shape for a specific experiment and to determine its parameters.
Fig. 4. Pulse shapes for $180^\circ$ inversion pulse G3, $90^\circ$ excitation pulse Q5, and $180^\circ$ refocusing Re-BURP pulses.
1.4.4 Pulsed field gradients

Pulsed field gradients (PFGs) are generated by so called gradient coils. The purpose of such coils is to generate a magnetic field that in addition to the standard homogeneous $B_0$ field, will generate a field over the sample of interest where field strength is dependent on spatial location. In essence, the effect of turning on a gradient is exactly similar to adding current to the $z^1$-shim coil. However as the gradient coils are specifically designed for this task, there are certain advantages to using them over the changing current in $z$-shim coil. Although many, if not all, gradient experiments are doable after a minor adjustment of replacing the current changes in gradient coils into current changes in $z^1$ shim coils.

The introduction of actively shielded gradient coils, which offer short recovery times for the re-establishment of the very homogeneous magnetic field after gradient pulse, made PFGs a routine tool in high resolution NMR [151, 152, 153, 154, 155]. By applying a gradient pulse (the strength of which is usually measured by gauss/cm, or tesla/m), one changes the magnetic field strength in the sample volume. This change in magnetic field strength is dependent on the spatial location along the sample and is usually adjusted to be linearly dependent on place. As the magnetic field strength is different along different parts of the sample, the Larmor frequency of spins is different in different parts of the sample. If we assume that all the spins in the sample at a certain time are pointing along the positive $x$-axis in the rotating frame, and we neglect the effects of $B_0$ and $B_1$ inhomogeneity, chemical shift and spin relaxation, for the time, without pulsed field gradient all the spins would stay coherent along the $x$-axis. As soon as the gradient pulse is turned on each of the spins will have their rotating frequency altered, if they are at the position where the gradient pulse changes the magnetic field strength. The longer the gradient pulse stays switched on, and the stronger the pulse is and the further apart the spins are from the coil center, the more different their rotating frequency will be. as a consequence spins lose their coherence and the intensity of the observable net magnetization drops very soon. After the gradient pulse has been switched off, the homogenous $B_0$ field is very soon present. At this point, each spin in the sample volume is once again rotating at the same frequency. The de-coherence developed during the gradient pulse has not, however, vanished. If, at any later point, one pulses another gradient with equal duration and intensity to the first one, but with inverted polarity, the Larmor frequencies in the sample volume are once again different, and dependent on position along the sample, but this time the spatial position changes the field strength in to the opposite direction compared to the previous one. If there has not been any diffusion present in the sample after this second pulse, all the spins are once again coherently aligned along the positive $x$-axis. When diffusion cannot be neglected, the experiment above can be used to quantify the amount of diffusion. This is because the spins that have changed position during the delay between the gradient pulses do not experience the same, but opposite, variation to the magnetic field by the gradient pulses, and do not add to the coherent signal detected [149, 150].

Another widely used way to use gradients is to first have the magnetization vectors made de-coherent by the first gradient pulse, have additional steps of pulse
Fig. 5. A few typical ways to utilize pulsed field gradients: In A, only the magnetization that is refocussed and inverted by the 180° pulses (open rectangulars) on I and S channels is rephased by the latter gradient. In B, the magnetization that is not parallel to the z-axis between the two 90° (filled rectangulars) pulses is defocused by a gradient pulses. In C, the transverse I spin magnetization coherence is defocused by the gradient pulse to be refocussed later in the pulse sequence. In the D sequence, solvent suppression is achieved by applying a train of selective pulses, each followed by a defocussing gradient prior to the 90° pulse.

sequence, and later in the sequence have another gradient pulse that would make the magnetization that have experienced exactly the pathway of coherence order manipulation required, coherent, but not the magnetization that has not. If the gradient pulse is left on and the acquisition is started, the received signal will have the spins resonating at a different frequency, dependent on their location. This is in essence how the magnetic imaging is done.

1.4.5 **Spatially selective spectroscopy**

Spatially selective spectroscopy is a form of spectroscopy in which the signal of interest is collected only from a part of the sample area. To achieve his, selective pulses and pulsed field gradients are usually needed. Although there are applications where neither of those are required, the high resolution spatially selective NMR spectrum can only rarely be obtained without the help of both of them.
The simplest way of acquiring a localized spectrum or image from a sample is by the use of a surface coil where, the localization is achieved by the strong \( B_1 \) field gradient of such coil [156]. By the use of carefully controlled gradients in the RF-field, more precise localization can be carried out [12, 157]. These techniques are not, however, applicable in standard high resolution NMR.

A more controllable acquisition environment in localized spectroscopy, and also in imaging, is obtained by the use of static field gradients. These gradients are primarily used as short phase encoding pulses, which label the spins at a particular position by giving them a well-defined RF phase. By collecting multiple FIDs, each with a different gradient, it is possible to mathematically reconstruct the spatial distribution of the spins [158].

![Figure 6](image)

Fig. 6. Selective pulse without \( B_0 \) gradient field does not provide any spatial selectivity (A). In presence of \( B_0 \) gradient field only those spins that precess at the frequency of the bandwidth of the selective pulse are affected. In B, only spins in the middle of the sample are affected by the selective pulse. After the \( B_0 \) gradient field is switched off spins once again precess at their nominal frequency. In C, the magnetization of the spins in the middle of the sample is, however, manipulated by the selective pulse.

1.4.6 Basic methods of spatially selective spectroscopy

One way of obtaining the spatial selectivity into data acquisition is by the use of Image-Selected In Vivo Spectroscopy (ISIS), where prior to acquiring a response from a non selective RF-pulse, a selective 180° pulse has been applied in the presence gradient field of each of the orthogonal gradient axes. By acquiring a series of 8 experiments with gradient pulses either switched on or off, and by calculating appropriate sums and differences of those experiments, a desired selective spectrum can be collected [159, 160, 161, 162].

Another method for which acquisition of several experiments is not required,
Fig. 7. Schematic representation of the ISIS sequence. Pulses along the proton (H:) and the three orthogonal gradient channels (g₁, g₂, and g₃). The filled rectangle represents a 90° non-selective pulse, the curved lines represent selective 180° pulses.

is the Point-Resolved Spectroscopy (PRESS). With PRESS the magnetization is first excited by a selective RF pulse in the presence of the gradient field along one dimension followed by a first echo time midway of which is a refocusing selective 180° pulse in the presence of the gradient field along the second dimension. This is then followed by a second echo time midway of which is again a refocusing selective 180° pulse in the presence of the gradient field along the third dimension [163, 164, 165, 166, 167].

A method not very much different from PRESS is the Stimulated Echo Acquisition Mode Spectroscopy (STEAM), where, instead of pair of refocusing selective 180° pulses, a pair of 90° pulses is used. The chosen magnetization is positioned along the z-axis during the delay between the two last pulses. By increasing the durations of the delays prior to and after the two last pulses, and the delay between those pulses, the resulting signals are weighted more by their $T_2$ or $T_1$ relaxation [168, 169, 170, 171, 172].
Fig. 8. Schematic representation of the PRESS sequence. The filled curved line represents a selective 90° pulse. For other explanations see Fig. 7.

Fig. 9. Schematic representation of the STEAM sequence. Symbols as in Fig 8.
2 Outline of the present study

Over the years the author had noticed some rather unexplainable behaviors with the repetitiveness of some of the published pulse sequences. In particular, some methods seemed to work better on one spectrometer, the others on another spectrometer. It was apparent that probe-head change could have an effect on the resulting spectrum, an effect larger than the difference between a reasonably and an extremely accurate pulse width calibration.

This inconsistency with the published and the actual results, even after careful acquisition set up, was especially pronounced when various water suppression techniques were compared. The general usability of a water suppression technique was not predictable by any clear theoretical means. Some methods seemed to have excellent performance with one hardware configuration but less than adequate with another, whereas another method could have a comparable performance with any configuration.

These observations led to a closer investigation of the not so clearly defined $\Delta B_1$, the RF-field inhomogeneity, that most of the time, unless specifically utilized, was assumed to be negligible in a modern NMR spectrometer. In order to achieve deeper understanding of the nature of this inhomogeneity, the use of spatially selective NMR techniques, usually used in the framework of MRS and MRI, was investigated together with the necessary modifications to the methods resulting from the different capabilities of the different instrumentation.
3 Materials and methods

3.1 Spectrometer

All the experiments in this work have been performed on a Bruker DRX-500 NMR spectrometer operating at a 11.7 T field. Mostly A 5mm BBI $^1$H-multinuclear probe-head equipped with shielded z-axis gradients has been used mostly. Occasionally a 5 mm TXI $^1$H-$^{13}$C-$^{15}$N probe-head, which is also equipped with shielded z-axis gradients was used. The spectrometer used was capable of producing 56 Gcm$^{-1}$ gradient strength. The gradient field strength was calibrated by using a sample of known dimensions and measuring an image of that sample along the z-axis. The measurement was repeated several times in slightly different sample positions and the mean value of the acquired image widths was used in gradient field strength calculation. The obtained value 56 Gcm$^{-1}$ is in excellent agreement with the gradient strength calibration results based on the diffusion measurements [173]. Only rectangular gradients were used, as the usage of any of the available procedures employing shaped pulsed gradients would have prevented the use of a gradient field during an applied RF-pulse. A 100 µs gradient recovery delay was used. The recovery delay was conservatively chosen to be about three times too long, as the effect of too short recovery delay would have been unacceptable. No pre-emphasis during the gradient recovery delay was used, as no improvement to the gradient recovery could be found, and as possible problems would have been introduced [174].

The spectrometer was equipped with the three separate RF-channels powered by 50, 100, and 300 W amplifiers for $^1$H, $^{13}$C, and $^{15}$N respectively. The obtainable $B_1$ field strengths were about 45.5, 20.0, and 7.8 kHz for $^1$H, $^{13}$C, and $^{15}$N, respectively. Proton field strengths were calibrated by the method presented in Chapter 4.1. The RF-field for the heteronucleus was calibrated by indirectly detecting the phase change of the proton satellite signals.
3.2 Acquisition

All the used pulse sequences were self written. An extra effort was put to assure enough time for spectrometer pulse phase, power, and gradient switching.

For selective pulse mainly Re-BURP refocusing pulse shape was used. Pulse shape was generated by the stdisp-program and defined by 1000 points. The excitation profile of the rephasing pulse was calibrated by increasing the selective pulse offset parameter value, and for the used 500 µs pulse an excitation bandwidth of 12 kHz was obtained. The boundaries of the excitation profile were taken to be the positions where less than half of the magnetization obtainable with the smaller offset was detected.

In the acquisition of the data for Section 4.1, the gradient power level was adjusted to 11.3 G/cm. No gradients were used in the acquisition of dataset without spatial resolution. A 2.9 s repetition rate of successive scans was used: this value is more than five times the relaxation time of the water line. Sixty-four increments of pulse length, each 1 µs, were recorded. The acquisition of 162 points was performed in 674 µs. The spectral width was adjusted to 125 kHz, corresponding to a 26.2 mm slice along the z-axis. An un-shifted sine bell was used as the window function, and the data was zero filled to 512 points before collected increments were subjected to Fourier transform. The total acquisition time for each experiment was 7 minutes.

The acquisition parameters for work in Section 4.2 are explained to some detail in the legends to the Fig. 15.

For the acquisition of $B_0$ field maps explained in Section 4.3, 1.9 s acquisition time and 2 s repetition rate, 100 Hz spectral width, and 1.4 mm voxel height was used for single acquisition. Typically voxel centers were 8.4 mm below and above the coil center in the first and last acquired spectrum, respectively, and the number of acquisitions were evenly spaced between these values.

The 1-D data presented in Section 4.4 was acquired by using a 1.25 s acquisition time and a 2 s repetition time. The localization parameters were empirically fine tuned for optimum voxel selection for each sample before the start of the actual data. No localization sequence was used in the acquisition of spatially non-selective data. Water suppression was achieved by modified CHESS sequence and used 56 applied selective pulses at water resonance frequency each followed by a 2 ms long 22 Gcm$^{-1}$ gradient pulse. The phase of every other selective pulse was inverted and the application power level was doubled. Before the acquisition the power levels of the pulses were independently adjusted manually for the smallest absorptive residual water signal. In 2-D data sets an acquisition time of 0.1 s and repetition rate of 1.2 s were used. A 40 ms long DIPSI mixing sequence was used with $B_1$ field strength of 7 kHz.
3.3 Processing

All the phase sensitive data were multiplied by an exponential window function, matched to the apparent $T_2$ relaxation time, as was also the acquisition time. In the experiments where acquisition of phase sensitive data was not possible, magnitude calculation of the resulting spectrum was conducted. In these cases a shifted sine-bell window function was used, and by manual adjustment of the phase shift of the window function, the resolution was maximized. If a series of experiments was done, slightly conservative values would be used to assure reliable signal position collection.

At least two time zero-filling was used, and in most cases the degree of zero-filling was even higher. A fifth degree polynomial base line correction was used in the spectra when required to flatten the baseline.

Signal to noise calculations were performed by adjusting the signal region to the desired range and by using a similar sized range for the noise region. Care was taken to exclude possible spurious artifact or sample contamination peaks outside both of the regions. Within a series of experiments, same regions were used exclusively.

3.4 NMR-tubes

Most experiments were performed using Wilmad 535-PP tubes, while a few experiments were performed using Wilmad 528-PP tubes. No difference between the outlook, apparent quality, or the obtained spectra between these two tubes was detected. In small sample volume work either a Shigemi-tube, a corresponding product from Cortec, separate susceptibility plugs in a standard sample tube, or self made plugs were used.

3.5 Samples

A series of Bruker test samples were used in setting up the experiments and acquiring the preliminary spectra, especially 2 mM sucrose in 90% H$_2$O sample. Doped water was used in the work explained in 4.1 and 4.3, and 820 mM saccharose in the D$_2$O sample in the work explained in 4.1 and 4.4. Samples whose preparations are explained in detail in [187] were used in the acquisition in 4.4.
3.6 Experimental setup

Prior to the acquisition of the new sample the probe head was carefully tuned and the $B_0$ homogeneity was adjusted, in most experiments by the method explained in 4.3.
4 Results and Discussion

4.1 RF-pulse calibration and the $B_1$ field homogeneity determination within a single experiment

In this part of the work, a method is presented, not only to calibrate the pulse length accurately, but also to obtain a value for the $B_1$ homogeneity of the probe-head, in a spatially encoded mode. The method proposed is based on a single image of the sample acquired. The signal collected in the presence of a single axis gradient, which for now is assumed to be along the z-axis, is

$$F(k) = \int f(z)e^{i\gamma Gt z}dz,$$  \hspace{1cm} (4.1)

where

$$f(z) = A(\Theta(z))\rho(z)\xi(z),$$  \hspace{1cm} (4.2)

is the signal originating from the sample with the nutation angle through $A(\Theta(r)) = \sin(\Theta(r))$, the spin density $\rho(r)$ and the coil responsiveness $\xi(z)$ \cite{29,30,32}. The resulting spectrum shows a one-dimensional image of the sample along the z-axis. Several experiments can be performed in a sequence with an increasing pulse width from one experiment to the next. When the obtained 2-D data matrix is Fourier transformed along the observable dimension only, the resulting spectrum shows a 2-D presentation of the $B_1$ field strength as a function of pulse length and probe coil position. Each column of the obtained 2-D spectrum has the same information along the xy-dimension as the traditional quantitative $I_{P_{\sin}}/I_{P_{90}}$ experiment without spatial information (Chap. 1.2.2). As long as the dimensions of the sample are larger than the receiver coil, the result of the acquisition of a simple image is characteristic of the $B_1$ field strength, since active volume is determined by coil, rather than by sample dimensions.

The image obtained shows the signal intensity as a function of the spatial position along the z-axis. The collected image shows the contributions of both transmitter and receiving coil $B_1$ field variations. Since the transmitter and receiving coils are the same in a typical high resolution NMR spectrometer probe-head configuration, the field strength variations from the nominal will appear as the square
of the variation in signal intensity at a certain position. By following the value for a 360° pulse along the z-axis one obtains the true \( B_1 \) field profile (Fig. 10). Although only the z-axis gradient version of the method is presented here, should there be need for a \( B_1 \) profile along another axis, the only modification required is the change of axis of the gradient field applied. If required, the experiment can easily be expanded to a multi-dimensional version, with more than one spatially resolved dimension. The two-dimensional experiment presented here is based on a simple spin echo experiment to obtain a one-dimensional image of the sample, with an additional dimension for increasing pulse width.

![Fig. 10. The result of \( B_1 \) profile mapping of a probe-head is shown. Separation of the lines is approximately 0.1 mm. The number of pulse length increments is shown along the horizontal axis. Experimental parameters were adjusted so that the pulse length used (in \( \mu s \)) was coincident with the number of increments. Data is presented in absolute value.](image)

The obtained results showed that the \( B_1 \) field values were quite even along the center of the coil, but decreased towards the edges. From a standard experiment without spatial resolution, one can obtain a \( B_1 \) field strength of 44.4 kHz for the whole probe-head. From an experiment with spatial resolution, the calculated \( B_1 \) field strength along the center part of the coil volume was 45.5 kHz (Table 1), beginning to drop at distances 5 mm from the center of the coil. Farther away from the coil center the field strength decrease was even more profound. An important point, however, is that an experiment without spatial resolution estimates the \( B_1 \)
field strength to be too low for the most parts of the sample volume, giving correct values for only a small part along the positions close to the coil edges, and too low for the outermost parts of the sample volume.

Fig. 11. Column extracts from experiments without (A, B) and with (C, D) spatial resolution. The data presented in A is presented in absolute value in B. C and D are extracts from the RF-coil center and from 6 mm below it, respectively. Both datasets have been acquired with similar parameters, except that no gradients were used during the acquisition of dataset without spatial resolution. Dashed lines show the position for the 360° pulse. The scale and pulse length are the same as in Fig. 10.
Like the $B_1$ field strength values, the percentage for the $P_{810}/P_{90}$ signal intensities are also obtained with spatial resolution. Such $P_{810}/P_{90}$ values will reveal the $B_1$ field homogeneity along the two other dimensions in which the gradient field has not been applied. Using only the z-axis gradient, the $B_1$ field homogeneity along the xy-dimension can be investigated. For the central parts of the sample volume, $P_{810}/P_{90}$ values above 90% were obtained (see Table 1). These values were significantly higher than the sum value of 73.7% obtained from the standard experiment without spatial resolution, and only slightly better than could be measured for a position 6.0 mm below the coil center, from where a value of 66.4% was obtained (Fig 11). At the edges of the active sample volume, neither $B_1$ nor the homogeneity along the xy-axis are sufficient for observing the intensity of the 810° pulse reliably in a reasonably short time with the method presented here. The number of experiments required depends on the increment of the pulse, the $B_0$ field strength, the $B_1$ field homogeneity and on how accurately one wishes to obtain the further values of magnetization rotation from the parts with the smallest $B_1$ field strength. As it appears, the areas with the smallest $B_1$ field strength also have the largest $B_1$ inhomogeneity, both of which increase the required experiment time significantly. The overall experiment time is mostly dependent on the $T_1$ relaxation properties of the sample, but in general, all the information mentioned above can be obtained from a single experiment that would take, as in this case, 7 minutes.
Table 1. $B_1$ field strengths, and $I_{P_{90\degree}}/I_{P_{90\degree}}$ and $I_{P_{110\degree}}/I_{P_{90\degree}}$ values obtained from the experiment shown in Fig 10. The spatial position of the 2-D column extract relative to the coil center is shown in the first column. For comparison, the last row shows the values obtained from the data shown in Fig 11 B.

<table>
<thead>
<tr>
<th>Position [mm]</th>
<th>$B_1$ [kHz]</th>
<th>$P_{450}/P_{90}$ [%]</th>
<th>$P_{810}/P_{90}$ [%]</th>
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<tbody>
<tr>
<td>-8</td>
<td>19.2</td>
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<td>-</td>
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<td>Trad. experiment</td>
<td>44.4</td>
<td>83.8</td>
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</table>

4.2 Increased sensitivity and improved spectral resolution through the use of localized spectroscopy.

The quality of the acquired signal can be improved, even to a significant extent, by not collecting any of the signal coming from the outer parts of the RF-coil. To achieve this a localized data acquisition was used. Throughout this part of the work a simple one dimensional modification of the basic localization methods used in MRS (Magnetic Resonance Spectroscopy) was used [163, 164, 181]. A selective refocusing 180° pulse in the presence of the applied $B_0$ field will refocus only that magnetization which rotates about the z-axis at the precession frequency matched to the excitation bandwidth of the selective pulse. Additional spectral purging can be achieved by exorcycling the selective pulse. Only the first $x,y$ part of the exorcycle phase cycle is required to achieve most of the purging efficiency of the phase cycle.
Fig. 12. Extracts of HSQC spectra of sucrose acquired from 1 mm high voxel at a) 0 mm, b) 2 mm, c) 4 mm and d) 6 mm below the coil center from top to bottom. The signal of the anomeric proton is shown. The intensity of the desired signal drops along with its line shape when the acquisition position is moved further from the coil center.
To demonstrate the advantage of using localized data acquisition, a spatially selective 1-D version of the well known HSQC [182, 183] experiment was performed. This experiment was chosen because the number of consecutive pulses within a sequence is rather high. In order to demonstrate the unwanted signal intensity dependence on the sample position, no phase cycling or pulsed field gradients were used to suppress the signal originating from the unwanted coherence pathway. The localized acquisition was achieved by positioning a selective refocusing 180° pulse in the presence of an applied field gradient prior to acquisition. The pulse lengths were set based on the method presented in 4.1, although the amount of unwanted magnetization could have been minimized by manual fine adjustment of 1H pulse lengths. However, since the intensity and the phase of the unwanted magnetization made the reliable signal intensity measurement of the desired magnetization practically impossible, a separate series of experiments along different positions of the vertical coil length was performed. In this set of data, a pair of pulsed field gradients was used to select the magnetization originating from 13C-bound protons, and in the latter experiment a minimal x, −x phase cycle of the last carbon 90° pulse, together with the acquisition phase cycling, was used. Otherwise the two series of experiments were identical. Two separate series of experiments were performed. One with 1 mm voxel height centered from -9 mm to +9 mm from the RF-coil center with 1 mm increments, and the second where the voxel center was kept at the coil center, but the voxel height was increased, in 1 mm steps, from 1 mm to 18 mm. The amount of the desired magnetization from the anomeric proton of the sucrose sample at 5.25 ppm, as well the amount of the unwanted magnetization from 12C-bound protons, was compared to the noise level for each of the acquired spectra (Fig. 12).
Table 2. Signal-to-noise ratios obtained at various voxels with 1 mm height along the z-axis. The value for the undesired magnetization is normalized to the desired magnetization by taking into account the different spin densities of the originating magnetizations.

<table>
<thead>
<tr>
<th>Position [mm]</th>
<th>S/N desired</th>
<th>S/N undesired</th>
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<td>7</td>
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<td>8.37</td>
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<tr>
<td>8</td>
<td>-</td>
<td>8.60</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>5.31</td>
</tr>
</tbody>
</table>
Fig. 13. Signal-to-noise ratios from 1-D HSQC spatially selective measurements at various parts of the sample volume. The voxel height was adjusted to 1 mm. The ratio of the observed signal to the noise is shown for the desired and undesired magnetization, using solid and dashed lines respectively.

Fig. 14. Signal-to-noise ratios for the desired magnetization from an experiment similar to that shown in Fig. 13, with an increasing active sample volume.
From the obtained signal-to-noise ratios (Table 2.), one can readily observe that the amount of desired magnetization relative to the noise is at its highest in the experiments acquired from the center parts of the coil. This remains rather high from positions 4 mm below the coil center to 6 mm above the coil center positions. Outside these regions, the amount of desirable magnetization drops quite quickly. The amount of undesired magnetization stays fairly constant in respect to the noise in all the experiments, except for those experiments from the largest distance from the coil center. Without relaxation and with perfect pulses, this magnetization should be positioned along the z-axis. Compared to the S/N ratio for the desired magnetization, it seems that about one half of the magnetization in the experiments acquired from the central parts of the coil is actually along the z-axis. In the outer parts of the coil, where the $B_1$ field strength is weaker, the amount of observable undesired magnetization does not drop, as the amount of desired magnetization does, both relative to the noise present.

As can be seen in the graphical presentation of the same data in Fig. 13, the amount of the collected desired magnetization clearly exceeds that of the collected undesired magnetization in the central portion of the coil, whereas the opposite is true for the edges of the coil. Because of this, it could be tempting to collect the signal only from the central part of the coil. However, the results from the experiment series with increasing voxel height show that the total signal-to-noise ratio still increases after the voxel height is greater than that of the center part of the coil, although the increase is clearly slower, as shown in the latter part of the curve in Fig. 14. The author wishes to point out that the use of more extensive phase cycling or a greater number of purging and magnetization selecting gradients would have eliminated virtually all of the unwanted signals.
Fig. 15. The pulse sequence used to obtain the 1-D HSQC spectra. Narrow and thick rectangles represent 90° and 180° pulses respectively. The curved line represents selective 180° pulse. A delay length of $1/(4J_{CH})$ was used for $\Delta$ and 1.3 ms for $\tau$. Gradients $g_1$ and $g_2$, with strengths 44.8 and 11.2 G cm$^{-1}$, respectively, and 1.2 ms durations were used to select the desired coherence pathway. The carbon pulse marked with $\phi$ was phase cycled x, -x together with the receiver x, -x phase cycle. Localization was achieved with a 500 $\mu$s Re-burp pulse during which a 28 G cm$^{-1}$ gradient ($g_3$) was applied for the 1 mm voxel height. Smaller gradient strengths were used in acquisition from higher voxel heights. No phase cycling or purging gradients were used in the collection of the undesired magnetization.
4.3 $B_0$ field map based on a series of localized 1-D NMR spectra

The method presented in this part of the work is based on acquisition of a $B_0$ field map by collecting a series of one dimensional spatially selective spectra displayed in the form of a 2-D spectrum. When a single line from each of the acquired spectra is displayed, the resonance frequency of the signal can be followed along the sample length and even small $\Delta B_0$ values can be observed. A z-axis-gradient-only spectrometer hardware configuration is assumed for the time being. The resolution of the displayed field map can be freely chosen and, if very high accuracy is required, numerous experiments can be run. In the map shown in Fig. 16, 128 1-D spectra were acquired and displayed. For faster experiment time, a map of only four to eight spectra would suffice, and relatively accurate shim setting changes can be derived from the resulting field map.

Fig. 16. The $B_0$ field map obtained by measuring 128 localized 1-D spectra at different locations along the vertical axis of the sample.
Fig. 17. The $B_0$ field profiles obtained by first adjusting the shims by $z_1 +500$, $z_2 +1000$, $z_3 +3000$, $z_4 +10000$, $z_5 +20000$, $z_6 +40000$ units in A, B, C, D, E, and F, respectively, from the situation shown in G.
As practically any liquid state NMR sample contains a proton signal, there is no need to use any other nuclei for the signal acquisition. If for some reason the use of proton acquisition is not satisfactory, the spatially selective acquisition can also be tuned to some other nucleus. If the spectrometer on which the method is to be utilized uses digital oversampling, thus disabling in practice all the signal folding, the method can easily be used to detect a sharp signal, even in the presence of a dominant signal, for instance water, without the folding of the intense signal outside of the spectral region. The use of organic solvents does not disable the usability of the method, as even the residual proton signal of the solvent is sufficiently strong to be used as the peak whose resonance is followed, usually even without the need for signal averaging in the different localized spectra acquired. In contrast to the aforementioned gradient shimming method (Section 1.3.3.), the signal choosen does by no means has to be a singlet, as all the signal shapes can be utilized in performing the field map, although the sharper the acquired signal, the easier it is to interpret the obtained results.

For the guidance of the effect of certain shim coils and for reasonable estimation of the required shim adjustment, a series of shim maps with deliberately miss-set shims was run, the results of which are shown in Fig. 17.

Based on the maps presented alone, a reasonably efficient shimming procedure can be derived. With the help of the maps, the shims that require adjustment can easily be spotted, and the magnitude of the required adjustment can also be estimated. However, the most efficient result can be obtained by combining the use of the acquired shim map, the shim setting offset of the previously run shim map and the direct response from the lock signal level. As the acquired homogeneity map shows distinct variation from the optimum, the shims that would correct the situation can be spotted from the acquired maps and the amount of change can be easily determined from the response in the lock signal. As the shims whose settings need adjustment can be directly located, the adjustment of individual shim can be several orders of magnitude higher at one time, if required, than in the standard procedure where no direct indication can be derived regarding which shim the inhomogeneity of the main magnetic field is coming from.

For instance, the field map shown in Fig. 18 requires adjustment of both the $z^3$ and $z^4$ shims simultaneously by several thousand units, both of which can be adjusted based on the result of a single homogeneity map acquisition, and following the lock signal increment as the shims are repeatedly adjusted. In particular, the shim adjustments resulting in the field map and spectrum in Fig. 18 A and B are good examples of a local optimum reached by the shim setting adjustment based on the lock signal and signal shape. From the shim settings resulting in the spectra shown, only a drastic adjustment of both $z^3$ and $z^4$, together with the patient adjustment of the lower shims would lead to a higher lock signal level. Since the line-shape in the upper 1-D spectrum is already reasonably good, it is unlikely that the substantial efforts required for reiterating the shim settings again would be made.

Results unobtainable by other shimming procedures have been achieved for such unusual samples as those where no deuterium lock could have been used, and where the proton content is so small that even the proton FID signal cannot clearly be
Fig. 18. The field map (A) and the corresponding 1-D spectrum (B) after reaching a local field homogeneity optimum by lock signal level optimization. By adjusting shims $z^3$ and $z^4$ by -3000 and +22000 units respectively, the situation in the lower field map (C) and spectrum (D) is obtained. In both spectra a sample of 0.8 M sucrose in D$_2$O was used. The Acquisition time was 2 s and a 0.5 Hz exponential window function was applied prior to Fourier Transform in both cases.
separated from the noise. For such a sample, shimming by the use of the standard method is very difficult, and indeed in practice, impossible. The method presented here has also been successfully used in shimming a gas sample for which regular shimming methods would be inefficient.
4.4 Sensitivity improvement by the usage of spatially selective acquisition

A clear view of the sensitivity gain obtainable using the methods presented in 4.1, 4.2, and 4.3 is obtained from the work presented in [187]. For a detection limit observation a series of chlorogenic acid sample dilutions was performed. A 50 nmol sample amount in 75 µl of 90%/10% H₂O/D₂O gave S/N of 9.5 with one pulse.

Fig. 19. Aromatic resonances of a 1-D water suppression proton spectrum of a 50 nmol chlorogenic acid sample.

From the same sample a 2-D TOCSY spectrum with recognizable cross peaks was acquired in 9 minutes. All the cross peaks seen in the spectra acquired with longer acquisition time from more concentrated sample were also visible in this spectrum. The line-shapes of both diagonal and cross peaks are exceptionally absorptive for a TOCSY experiment. No phase line roll or artifact ridges are present in either dimension.

The lowest drawn contour level in the spectrum is slightly higher than the spectral noise level.

From a sample of 3.7 nmol, a S/N of 4.92 was achieved with 128 scans. The line shape of the peaks is very good in this experiment also with no signal broadening due the small sample height, which was approximately 5.5 mm. The adjustment of the B₀ field homogeneity was also straightforward when using the method presented in Section 4.3. The required amount of adjustment of the shim currents is, however, rather substantial. The achievement of a high resolution spectrum without the assistance of the shimming method used would have been highly unlikely, although only the shims z¹, z², x, and y have an effect along the sample volume this minuscule.
Fig. 20. The aromatic region of 2-D TOCSY spectrum of acid 50 nmol chlorogenic acid sample.

Fig. 21. Aromatic resonances of a 1-D water suppression proton spectrum of a 3.7 nmol chlorogenic acid sample.
The spectrum from a sample amount of 225 pmol gave a S/N of 63 with 45274 scans. The origin of the strong artifact doublet at 5.35 ppm is unknown, but it is possible that the signal is also present in the spectra from larger sample quantities but is not seen due its low intensity. This signal is however excluded from the signal-to-noise-ratio calculations.

The $B_0$ field homogeneity is still very good. No deviation from the ideal line shape can be seen for this spectrum either.

For comparison purposes a signal to noise ratio from a 1.2 µmol sucrose sample was run (Standard Bruker water suppression test sample with 2 mM sucrose in 90%/10% $H_2O/D_2O$). All the acquisition parameters were identical to those used in the acquisition of spectrum in Fig. 19, except that no localization sequence was used. An experiment with two pulses was run as the baseline distortions after one pulse were too severe for meaningful signal-to-noise-ratio calculations. A flat baseline is observed after two pulses as a result of a two step phase cycle combined with the polynomial baseline correction.

An S/N figure of 63 was obtained. The smaller S/N ought to come from the lower quality of the water suppression that disables the use of a receiver gain setting optimized to the solute signal. The reason for this most probably lies in the lesser $B_1$ field strength at the edges of the active sample volume, as presented in Section 4.1.

A 2-D TOCSY spectrum similar to that in Fig. 20, but without the localization sequence, was also run. All the other acquisition parameters were identical, as were the contour levels in the displayed spectrum, relative to the noise level.

Although the sample amount was 24 times higher than in the acquisition of the spectrum shown in Fig. 20, there are numerous missing cross peaks. In addition

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Fig. 22. Aromatic resonances of a 1-D water suppression proton spectrum of a 225 pmol chlorogenic acid sample.
Fig. 23. A 1-D water suppression proton spectrum of a 1.2 $\mu \text{mol}$ sucrose sample.

The anomeric proton signal at diagonal is not seen, most of the signals show significant amounts of dispersive nature, there are artifact ridges along the indirectly detected dimension as well as along the $f_2$ dimension at the water frequency in $f_1$, and the rolling baseline disallows efficient spectrum phasing.

Fig. 24. A 2-D TOCSY spectrum of 1.2 $\mu \text{mol}$ sucrose sample.
4.5 Examples of presented methods in practice

4.5.1 Sensitivity and resolution study by spatially selective acquisition

For studying the optimal sample volume, as well as the optimal voxel height for the best obtainable signal amount and lineshape, a series of experiments was performed. For that purpose 20 mg of cholesterol was dissolved in a small amount (195 µl) of deuterated chloroform. From that sample a series of 2-D TOCSY and 1-D proton spectra was run, varying the height of the spatial voxel from which the signal was acquired (1mm, 2mm, 4mm, 6mm, 8mm, 12mm, and 16mm). Next, an approximately similar amount of solvent was added to the sample tube, and after carefully adjusting the homogeneity, similar experiments were run. The addition of solvent, shimming, and the series of acquisitions were performed twice more.

Specific consideration was given to adjusting the $B_0$ field to be as homogenous as possible. Still, for reasons yet unknown, the $B_0$ field homogeneity adjustments after the first addition of the solvent were unsuccessful. The $\Delta B_0$ along the sample volume was too large to see any practical difference in the lock signal level, acquired signal or $B_0$ field profile, no matter what kind of shim setting adjustments were attempted. No such difficulties were met with homogeneity adjustments with the other sample volume amounts. The author, however, has previously experienced similar occurrences and would speculate that, in such cases, the magnetic susceptibility of the sample affects the $B_0$ field strength distribution to such a degree that previously used shim coil current values are no longer even close to the optimal. Careful screening of the shim value space should reveal the shim currents that provide tolerable starting values for more organized homogeneity adjustment procedures. In this case, however, the after three hours of unsuccessful shim value screening, the same experiments as with the other sample volume heights were performed. As the resulting spectra do not qualify as high resolution NMR spectra, the S/N and linewidth values obtained from those experiments are excluded from the tables and figures presented in this chapter.
Fig. 25. Two 2-D TOCSY spectra of a 14 mm high sample volume of 52 µmol of cholesterol. The upper spectra is acquired from a 6 mm tall voxel, the lower one is acquired from a 16 mm tall voxel. Improved spectral quality by acquiring signal only from central part of the sample is evident from the upper spectrum.
The signal-to-noise figures, as well as the lineshapes, varied noticeably even within the acquisition series taken from the same sample but using varying localization voxel, as can be seen from Fig. 25. When the signal is collected only from the central 6 mm tall voxel, the amount of signal compared to noise is clearly greater than in spectrum in which the signal is collected from a 16 mm tall voxel. With the smaller voxel the $\Delta B_0$ is also much smaller, resulting in a significantly better lineshape along both of the orthogonal dimensions of the spectrum.

In the 1-D proton spectra, the S/N values were calculated from the resonance of proton 3 at 3.55 ppm, and in the 2-D TOCSY spectra from the same signal from the row of resonances of both protons 1 and 2, at 1.85 ppm.

![Chemical structure of Cholesterol, with numbering.](image)

Fig. 26. Chemical structure of Cholesterol, with numbering.

The S/N values obtained from the 2-D spectra reveal that the largest amount of signal, relative to the noise, is collected from the shortest, i.e. 14 mm tall sample when signal is acquired only from the central 6 mm voxel of its height. An almost similar S/N value is obtained for the acquisition from 12 mm localization height, but further increase in localization voxel height reduces the signal amount obtained, relative to the acquired noise. Acquisition of the signal from the whole active volume results in a signal-to-noise that is less than one third of what could be achieved by acquiring only from the smaller voxel.

When performing similar acquisitions using almost three times the sample volume, the maximum obtained S/N value is almost as large as the previous value, except that it is obtained by acquiring the signal from the whole active sample volume. Further increase in sample volume clearly diminishes the achievable signal-to-noise value, as can be seen from Table 3 and from Figure 27.
Table 3. S/N values obtained from rows extracted from acquired Tocsy spectra with varying localization voxel height and sample volume, while maintaining constant sample amount.

<table>
<thead>
<tr>
<th>Localization voxel height [mm]</th>
<th>Sample height [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>1</td>
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<td>12</td>
<td>111.7</td>
</tr>
<tr>
<td>16</td>
<td>49.9</td>
</tr>
</tbody>
</table>

Fig. 27. A 3-D representation of the obtained S/N from 2-D TOCSY as a function of both sample volume and localization voxel height.
The signal linewidths were determined by deconvoluting a Lorentzian lineshape over a singlet resonance of proton 18 at 0.72 ppm, and have practically no variation as a function of the localization voxel height. The experiments run from 39 mm and 52 mm high samples show no noteworthy changes in linewidth from each other. In the experiments run the from shortest sample, 14 mm tall, the linewidth is more than two times greater than in the other two experiment series. A clear reason for this is not known. The measured $B_0$ profile (see Appendix A) is quite straight, but it is possible that the off axis shims were not adjusted to the optimum, as the profile seemed to be quite thick. Another explanation for the larger linewidth could be higher sample viscosity in the sample with a smaller volume of solvent. As the spectra presented in section 4.4 show, the shortness of the sample itself is not the reason for linewidth increase. In those experiments, shorter samples produced improved lineshape and narrowed linewidths, therefore the shortness of the sample itself is not the reason for linewidth increase.

Table 4. Linewidth values obtained from rows extracted from acquired TOCSY spectra with varying localization voxel height and sample volume, while maintaining constant sample amount.

<table>
<thead>
<tr>
<th>Localization voxel height [mm]</th>
<th>Sample height [mm]</th>
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<tbody>
<tr>
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<tr>
<td>8</td>
<td>8.3</td>
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<tr>
<td>12</td>
<td>9.0</td>
</tr>
<tr>
<td>16</td>
<td>8.7</td>
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</table>

The corresponding S/N figures obtained from one dimensional proton spectra are somewhat different than those from TOCSY spectra, as can be expected based on the greater $\Delta B_1$ sensitivity of the latter resulting from the greater amount of 180° pulses used. The S/N figures are somewhat smaller in the experiments run from shortest sample height, and approximately equal in the spectra run from the two taller samples. Interestingly, the S/N values are quite even for the localization voxel heights from 2 mm to 16 mm, even though one could have expected an increase as a function of increased spins in the volume from which the signal was collected.
Fig. 28. A 3-D presentation of the linewidth obtained from rows extracted from acquired TOCSY spectra as a function of both sample volume and localization voxel height.
Table 5. S/N values obtained from acquired 1-D proton spectra with varying localization voxel height and sample volume, while maintaining constant sample amount.

<table>
<thead>
<tr>
<th>Localization voxel height [mm]</th>
<th>Sample height [mm]</th>
<th></th>
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<tbody>
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<tr>
<td>16</td>
<td>27.6</td>
<td>39.0</td>
<td>37.3</td>
</tr>
</tbody>
</table>

Fig. 29. A 3-D presentation of the obtained S/N obtained from acquired 1-D proton spectra as a function of both sample volume and localization voxel height.
The linewidths in the 1-D spectra do increase as a function of increasing localization voxel height, but only in the spectra run from the 14 mm tall sample. In the spectra run from the 39 mm tall sample, a small increase in linewidth with increasing localization voxel height can be seen, but in the spectra run from the 52 mm tall sample no notable changes in linewidth can be seen.
Fig. 30. A 3D presentation of the obtained linewidth as a function of both sample volume and localization voxel height.

Table 6. Linewidth values obtained from acquired 1-D proton spectra with varying localization voxel height and sample volume, while maintaining constant sample amount.

<table>
<thead>
<tr>
<th>Localization voxel height [mm]</th>
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<th>39</th>
<th>52</th>
</tr>
</thead>
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<tr>
<td>16</td>
<td>23.7</td>
<td>2.4</td>
<td>2.3</td>
</tr>
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</table>
4.5.2 Homogeneity adjustment with sample without deuterium lock.

For showing the competence of the shimming method presented in section 4.3, 2g of sucrose were dissolved into 3 ml of tap water and 400 µl of this was placed into a standard NMR tube. The initial $B_0$ profile and the spectra, shown in Figure 31, clearly shows that the $B_0$ field homogeneity was not very good. As no lock signal based shimming procedures could be used, there were no gradshim maps for gradient shimming by the Bruker gradshim-program, and the viscosity of the sample together with the radiation damping by the strong proton signals made it impossible to improve the shim settings by monitoring the shape and size of FID signal. Therefore, no traditional way of improving the $B_0$ field homogeneity existed. By acquiring $B_0$ field maps, and based on the information obtained, the shim values could be adjusted. Figure 32 shows the $B_0$ field profile after the first shim adjustment, together with the resulting proton spectrum. Further shim adjustments improved the $B_0$ field homogeneity even more, as can be seen from the top of Figure 33. The resulting spectrum shows symmetric lineshapes and no visible line broadening from $\Delta B_0$. Figure 34 shows the quality of water suppression with the sample, as well as the desired and undesired signals anomeric proton from 1-D HSQC spectra run in a similar manner as in Section 4.2.
Fig. 31. The acquired $B_1$ profile and initial $B_0$ profile together with the corresponding proton and water suppression spectrum.
Fig. 32. The $B_0$ profile after adjusting shims $z_1$ and $z_2$ by +500 units, both together with corresponding proton spectrum. The $B_0$ profile after further adjustment of $z_1$ shim by +200 units and another $B_0$ profile after adjusting shim $z_3$ by -500 units.
Fig. 33. The $B_0$ profile after adjusting shims $z^3$ and $z^2$ by -500 and -200 units respectively. The $B_0$ profile after adjusting $z^4$ shim by +2000 units, together with the corresponding proton spectrum and an its expansion.
Fig. 34. The water suppression proton spectrum after a $B_0$ field adjustment with expansion of the spectrum. The HSQC spectra of the desired and undesired signal, acquired in a similar manner as in Section 4.2.
5 Conclusions

5.1 $B_1$ field map

By using the method presented in Section 4.1, the $B_1$ field strength along one axis, typically the $z$-axis, can be easily mapped. Based on the information gathered from a single experiment, one can obtain reliable and valuable information about the $B_1$ field distribution, e.g. homogeneity of the coil. From such information, the degree of artifact suppressing methods, such as phase cycling or the use of pulsed field gradients required, for successful NMR experiments can be determined. Since normal pulse length calibration also requires the acquisition of several 1-D spectra, the required experimentation time is not increased by using the method described here. This method may also be routinely run on several hardware configurations, and rescans can be easily done at will.

5.2 Localized spectroscopy

The maximum amount of signal from an NMR experiment is obtained when the signal is acquired from a maximum number of resonating spins [184, 185, 186]. The results presented show that a significantly more homogenous $B_1$ field along the active sample volume is achieved by rejection of the signal originating from the outer parts of the coil length. Although the total amount of signal obtained from the outer parts of the RF-coil is not very high, some loss of signal is associated with the spatially selective acquisition. The rejected signal, however, is a significant source of artifacts, and if no precautions were taken, the artifacts would severely decrease the quality of the acquired data. If the sample concentration can be increased, it would be advantageous to dissolve the amount of sample available in as small an amount of solvent as is possible, and place the sample in the most $B_1$ homogenous part of the probe-head RF-coil. With the same amount of nuclear spins concentrated into a smaller volume, the sensitivity of an NMR experiment can be increased manifold. Care should be taken to acquire the signal only from such a
volume of the sample that does not suffer from the adverse effect of susceptibility incontinuities.

The work presented in Ref.[31] can also be used to obtain somewhat similar results. In this technique, the magnetization that is excited by the nominal 180° selective pulse in the presence of an applied gradient is destroyed prior to the start of the actual pulse sequence.

The disadvantage of collecting the signal only from the concentrated part of the sample by the method presented in this work is the loss of signal by relaxation during the time of the localization part of the pulse sequence. As the relaxation during the selective part of the pulse sequence is, in most cases, negligible apart from the fastest relaxing samples, the signal loss can be considered to be non-existent. Although, with use of the localization method in which spatial selection occurs before the actual pulse sequence, this problem can be avoided.

5.3 $B_0$ homogeneity

A method capable of producing a map of the $B_0$ field strength and its variation along the sample volume is presented. Similar results can also, in the optimal cases, be obtained more elegantly from a pair of 1-D imaging experiments with different echo times [35, 36, 37, 39, 40, 41, 42]. However, the image based $B_0$ field mapping is usable only when certain prerequisites are fulfilled, and even in these optimal conditions the time consumption of the image based methods is not significantly smaller than with the method presented here. On the other hand, the method presented here can be used with practically all kinds of liquid and gas phase NMR samples, even with samples of a solely deuterated solvent of those not having a strong single proton line. The usability of the method does not depend on the $\Delta B_0$ along the sample length, rather, the same method can be used for accurate fine tuning of the sample, usually without drastic acquisition parameter changes.

5.4 Sensitivity improvement

The improved sensitivity by the use of the localized acquisition is presented in Table 7. The results show an S/N figure per single acquisition to be from 100 to over 200 units per µmol of sample. With the standard acquisition of all the obtainable signal from long sample, results in a significantly lower sensitivity.

The latest trend in sensitivity improvement in high resolution NMR is, in addition to increasing the $B_0$ field strength, the use of cryogenic probe heads [188]. More than four-fold sensitivity improvements have been reported. However, the reported S/N figures are calculated from the sample in the active probe area. Not from the real sample quantity.

With the localized acquisition a sensitivity improvement of a similar magnitude
as that of the cryogenic probe head can be achieved. However, these same localized techniques can be utilized with the cryogenic probe heads as well, and even greater sensitivity gains can be obtained.

Table 7. Calculated signal-to-noise ratios from water suppression experiments, normalized to the single acquisition from a 1 µmol sample amount. Based on the presented S/N results obtained for 0.1% EB with a BBI and cryogenic probe head \([188]\), the corresponding result for cryogenic probe head was calculated.

<table>
<thead>
<tr>
<th>Sample</th>
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<th>Number of scans</th>
<th>S/N</th>
<th>Normalized S/N</th>
</tr>
</thead>
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<td>Estimated result with cryogenic probe head</td>
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S/N test results

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Bibliography


Fig. 35. $B_1$ and $B_0$ profiles a of 14 mm high sample volume of 52 µmol of cholesterol (Section 4.5.1).
Fig. 36. Spectra of 14 mm high sample volume of 52 µmol of cholesterol. The localization voxel heights used were 1 mm, 2 mm, 4 mm, 6 mm, 8 mm, 12 mm, and 16 mm. Proton spectra on left column and row of 2D-TOCSY spectra (Section 4.5.1).
Fig. 37. $B_1$ and $B_0$ profiles a of 39 mm high sample volume of 52 µmol of cholesterol (Section 4.5.1).
Fig. 38. Spectra of 39 mm high sample volume of 52 µmol of cholesterol. The localization voxel heights used were 1 mm, 2 mm, 4 mm, 6 mm, 8 mm, 12 mm, and 16 mm. Proton spectra on left column and row of 2D-TOCSY spectra (Section 4.5.1).
Fig. 39. \( B_1 \) and \( B_0 \) profiles of 52 mm high sample volume of 52 \( \mu \)mol of cholesterol (Section 4.5.1).
Fig. 40. Spectra of 52 mm high sample volume of 52 µmol of cholesterol. The localization voxel heights used were 1 mm, 2 mm, 4 mm, 6 mm, 8 mm, 12 mm, and 16 mm. Proton spectra on left column and row of 2D-TOCSY spectra (Section 4.5.1).