In-vivo Proton Magnetic Resonance Spectroscopy:

Evaluation of Multiple Quantum Techniques for Spectral Editing

and a Time Domain Fitting Procedure for Quantification
In-vivo Proton Magnetic Resonance Spectroscopy:

Evaluation of Multiple Quantum Techniques for Spectral Editing

and a Time Domain Fitting Procedure for Quantification

Proefschrift

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus,
prof. drs. P.A. Schenck,
in het openbaar te verdedigen
ten overstaan van een commissie
aangewezen door het College van Dekanen
op maandag 7 oktober 1991 te 14.00 uur

door

Johannes Edwinus van Dijk

geboren te Zwolle
electrotechnisch ingenieur
Dit proefschrift is goedgekeurd
door de promotors: prof. dr. ir. A.F. Mehlkopf.

## CONTENTS

1. Introduction  
   1. General  
2. Spectral editing and data processing techniques  
3. Editing techniques  
4. Multiple quantum coherences  
5. Data processing techniques  
6. Outline of this thesis  
7. References  

2. Comparison of double and zero quantum nmr editing techniques for in-vivo use (paper).

   Abstract  
1. Introduction  
2. Editing techniques  
3. Multiple quantum coherences  
3.1. Double quantum coherence sequences  
3.1.1. Selection of double quantum coherences by gradient pulses  
3.1.2. Double quantum signal enhancement by a selective read pulse  
3.1.3. Selection of double quantum coherences by phase cycling  
3.2. Zero quantum sequences  
3.2.1. Selection of zero quantum coherences by a gradient pulse  
3.2.2. Selection of zero quantum coherences by phase cycling  
3.3. Combined zero and double quantum coherences  
4. Suppression of unwanted resonances  
5. $B_0$ field inhomogeneity effects  
6. $B_1$ field inhomogeneity effects  
7. Localization combined with multiple quantum sequences
## 3. A localized in-vivo detection method for lactate using zero quantum coherence techniques (paper).

<table>
<thead>
<tr>
<th>Abstract</th>
<th>52</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>53</td>
</tr>
<tr>
<td>2. Principles</td>
<td>54</td>
</tr>
<tr>
<td>3. Results and discussion</td>
<td>57</td>
</tr>
<tr>
<td>4. Conclusions</td>
<td>60</td>
</tr>
<tr>
<td>5. References</td>
<td>61</td>
</tr>
</tbody>
</table>

## 4. Double quantum- and spin echo sequences for the selective detection of glutamate and glutamine (paper).

<table>
<thead>
<tr>
<th>Abstract</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>64</td>
</tr>
<tr>
<td>2. Definition of the spin systems</td>
<td>67</td>
</tr>
<tr>
<td>3. Experimental</td>
<td>70</td>
</tr>
<tr>
<td>4. Results</td>
<td>72</td>
</tr>
<tr>
<td>4.1. Double quantum editing sequences</td>
<td>72</td>
</tr>
<tr>
<td>4.2. Spin echo sequence</td>
<td>76</td>
</tr>
<tr>
<td>5. Discussion</td>
<td>78</td>
</tr>
<tr>
<td>6. Conclusions</td>
<td>80</td>
</tr>
<tr>
<td>7. References</td>
<td>82</td>
</tr>
</tbody>
</table>

## 5. A $^1$H-NMR spectroscopy study on the possible protective effect of L-carnitine on hyperammonemia induced encephalopathy in rats (paper)

<table>
<thead>
<tr>
<th>Abstract</th>
<th>86</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>87</td>
</tr>
<tr>
<td>2. Materials and methods</td>
<td>88</td>
</tr>
<tr>
<td>2.1 Animals</td>
<td>88</td>
</tr>
<tr>
<td>2.2 EEG spectral analysis and clinical grading</td>
<td>88</td>
</tr>
</tbody>
</table>
6. Determination of concentrations by time domain fitting of proton NMR echo signals using prior knowledge (paper)

   Abstract
1. Introduction
2. Introduction of the theory
3.1 Incorporation of prior knowledge
3.2 Prior knowledge about amplitudes
4. Experimental verification
4.1 Obtaining prior knowledge in-vitro
4.2 Using prior knowledge in-vitro
4.3 Adaptations of prior knowledge for in-vivo use
4.4 Results of the in-vivo measurements
4.5 Discussion
5. Conclusions

Appendix 1. Prior knowledge about frequencies
Appendix 2. Prior knowledge about damping constants

References

7. Summary and conclusions
1. General
2. Multiple quantum editing techniques
3. Data processing techniques

Samenvatting en conclusies
Concise list of abbreviations
CHAPTER 1. INTRODUCTION

1 GENERAL.

In recent years Nuclear Magnetic Resonance (NMR) has proven to be useful for medical purposes. The first application of Magnetic Resonance Imaging (MRI) was published in 1973 (1). The first publication of Magnetic Resonance Spectroscopy (MRS) on living tissue was presented in 1974 (2). Especially MRI, which makes it possible to produce images of the water distribution is widely used in the clinic. The importance of MRS grew continuously in the last ten years. With MRS metabolic processes can be studied non-invasively in-vivo, since several metabolites can be detected. Not only phosphorus but also carbon, sodium and especially proton MRS are commonly used. In this thesis, only aspects of proton MRS are discussed. Especially for proton NMR it is important to edit and to have sophisticated data processing techniques because of reasons mentioned below. Although not mentioned, many descriptions are in principle applicable to other nuclei as well.

The application of NMR in-vivo is especially attractive from the medical point of view but several physical problems are encountered:

1) signals are detected from many metabolites whose spectral components are within a relatively small frequency region,
2) due to differences in susceptibility within the tissue, the static field inhomogeneity, and relaxation effects, the peaks in the spectrum are broadened,
3) artifacts due to motion and system instabilities must be prevented. Therefore large water and lipid signals must be suppressed,
4) the dynamic range of the apparatus is limited (3). This also means that large water and lipid signals must be suppressed in order to detect signals from metabolites which are present in low concentrations,
5) volume selective detection is in general necessary,  
6) the signal to noise ratio is poor.  

Due to the aspects mentioned at 1 and 2, the spectrum may consist of  
many, partly overlapping peaks. Observation of signals from  
metabolites and quantification of their concentrations, which is of  
great importance for the study of metabolic processes, is often  
difficult because of this overlap. A possible solution of this problem  
is to measure selectively signals from specific metabolites with  
editing techniques. If this does not completely solve the problem,  
sophisticated data processing techniques can be used to analyze the  
measured signal. In this thesis both approaches are discussed.

2 SPECTRAL EDITING AND DATA PROCESSING TECHNIQUES.

Using editing techniques (4,5,6,7), spectra can be obtained with peaks  
of specific metabolites while signal contributions of other  
metabolites are suppressed. This can either be done by addition and  
subtraction of signals from different measuring sequences or by  
measuring selectively certain signal components. In general, the  
editing sequences have the advantage of giving relatively simple  
spectra. On the other hand three disadvantages can be given:  
1) In most cases it is necessary to perform more than one measurement  
   if information about more than one metabolite is required.  
2) The signal to noise ratio usually is lower as compared to  
   non-editing sequences. In in-vivo MRS the signal to noise ratio is  
   already poor.  
3) Editing sequences that are based on subtraction techniques are  
   sensitive to motion and instrumental instability.

Despite these disadvantages, editing techniques are often the only  
choice. A well known example is the lactate signal which in tissue  
containing lipids, can only be detected if the lipid (CH$_2$)$_n$ signal is  
eliminated.
In general, but especially if the available measuring time is limited, a non-editing sequence which gives signals from all metabolites is favorable. Data processing techniques (8,9,10,11) however, must then be able to differentiate between the signal components of the metabolites. For this reason it is important to develop sophisticated data processing techniques which are able to analyze the measured NMR signals. However, since it is unlikely that all overlapping peaks can be accurately quantified by data processing techniques, it is also worthwhile to develop editing techniques.

3 EDITING TECHNIQUES.

Editing sequences are in this thesis defined as only those sequences that yield a signal in which contributions of one or more metabolites are present and at least one signal contribution of one metabolite, not being water, is suppressed. The water suppression is dealt with as being one of the aspects related to in-vivo MRS. It must be stressed that an editing sequence is always based on some difference between the compounds to be measured and the ones to be suppressed. This can be a difference in relaxation time, coupling constants- and networks, chemical shifts, and multiple quantum frequencies. For in-vivo studies the following demands must be fulfilled:

1) The water signal must be suppressed or, if the editing sequence itself does not give a good water suppression, it must be possible to combine the editing sequence with a water suppression method.

2) It must be possible to combine the sequence with localization techniques.

3) Motion artifacts must be prevented. This means that phase cycling schemes must be avoided as much as possible.

4) The loss of signal to noise ratio must be as small as possible since for most in-vivo studies long measuring times are unacceptable.
In the introduction of chapter 2 the relevant editing techniques for in-vivo NMR are mentioned. In this thesis, one of the promising editing methods, the one based on multiple quantum coherence techniques, is investigated. The most important advantage of multiple quantum editing techniques is that these have an additional parameter to distinguish signals from different metabolites, which is the modulation corresponding to the multiple quantum frequencies. The aim of the chapters about multiple quantum editing techniques is to show how editing sequences based on multiple quantum coherence techniques, can be used for in-vivo studies to measure specific metabolites which otherwise could not or only inaccurately be detected.

4 MULTIPLE QUANTUM COHERENCES

In this section the definition of multiple quantum coherences (mqc's) is given and some important properties of these are discussed. For a more detailed description the reader is recommended to study chapter 2 of this thesis or chapter 5 of reference (12). For simplicity a two spin AX system is considered. The spins A and X are weakly coupled with scalar coupling constant J. In figure 1 the energy level diagram and the spectrum are shown. The energy levels are expressed in frequency units. The quantities $\omega_A$ and $\omega_X$ are the resonance frequencies of the spins A and X respectively:

$$\omega_{A,X} = \gamma B_0 (1 - \sigma_{A,X}), \quad \sigma_{A,X}$$

being the screening constant of nucleus A or X.

In the broadest definition, mqc's are those coherences that correspond to a change of the total magnetic quantum number not equal to plus or minus one. This total change of magnetic quantum number is also called the order of the mqc. With this definition, a coherence between the levels $E_1$ and $E_4'$, being a double quantum coherence and a coherence between the levels $E_2$ and $E_3$, being a zero quantum coherence are considered to be multiple quantum coherences. If the spin system
consists of more than two spins, coherences of orders higher than two can be present and it is necessary to define the term \( q \)-spin-\( p \)-quantum coherence. A \( q \)-spin-\( p \)-quantum coherence is of order \( p \) where \( q \) is the number of actively involved spins. Note that for the two spin system

\[
E_1 = \frac{1}{2} (\omega_A + \omega_x) + \frac{1}{4} J
\]

\[
E_2 = \frac{1}{2} (\omega_A - \omega_x) - \frac{1}{4} J
\]

\[
E_3 = \frac{1}{2} (- \omega_A + \omega_x) - \frac{1}{4} J
\]

\[
E_4 = - \frac{1}{2} (\omega_A + \omega_x) + \frac{1}{4} J
\]

\[
\begin{align*}
E_{24} & \quad E_{13} \\
E_{34} & \quad E_{12} \quad \rightarrow E
\end{align*}
\]

Figure 1. The energy level diagram and the NMR spectrum of a two spin system with weak scalar coupling \( J, \sigma_A > \sigma_X \).

four single quantum coherences are possible but only one zero and one double quantum coherence can be present. This is one of the principles used by multiple quantum editing techniques to simplify the NMR spectrum. The single quantum coherences give signals with frequencies:

\[
E_1 - E_2 = \omega_x + J/2,
E_1 - E_3 = \omega_A + J/2,
E_2 - E_4 = \omega_A - J/2,
E_3 - E_4 = \omega_x - J/2.
\]

The double and zero quantum frequencies of the two spin system are given by

\[
E_1 - E_4 = \omega_A + \omega_x \quad \text{and}
E_2 - E_3 = \omega_A - \omega_x.
\]
The basic scheme of a mqc sequence is shown in figure 2. During the preparation, the mqc's are excited. These freely precess during the evolution period and are transferred into observable transverse magnetization during the mixing period. The detectable magnetizations can be measured in the detection period. MQC's are not directly observable but the multiple quantum frequencies can be recognized by studying the detected signal as a function of the evolution time.

| preparation | evolution | mixing | detection |

Figure 2. The basic scheme of multiple quantum sequences following the definitions of Ernst et al (12).

The phases of the preparation pulses influence the phase of the created mqc's. A change $\phi$ in the phase of the creation pulses results in a phase shift $\rho \phi$ for the $\rho$ quantum coherences. This property can be used to separate different orders of mqc's (13,14).

Another important and often used property of mqc's to select a certain order is based on the effect of pulsed field gradients. This effect is in detail described in references (15,16). A gradient pulse dephases a $\rho$ quantum coherence $\rho$ times as much as a single quantum coherence. The dephased mqc's which are converted into transverse magnetizations can be rephased by a $\rho$ times longer (or stronger) gradient pulse. Coherences of other orders are not rephased after the two gradient pulses. Therefore only mqc's of order $\rho$ contribute to the detected signal. This technique is preferable to phase cycling because motion artifacts are prevented.
5 DATA PROCESSING TECHNIQUES.

Before data processing techniques can be applied, several aspects must be considered:

1) For NMR studies, the measurement domain is the time domain and the transformation domain the frequency domain. Transformation from the time to the frequency domain can be performed by fourier transformation. Applying data processing in the time domain avoids preprocessing of the data to transform them to the frequency domain. A well known problem of quantification in the frequency domain is a distortion of the spectrum because of an incomplete data set. Truncation of the data set usually does not affect time domain quantification methods.

2) In the chosen domain a model function must be given which can be used by a fitting procedure. Quantification methods without fitting can be used (17,18,19) but only fitting techniques will give the optimum results for in-vivo measurements because the spectra of these signals consist of many overlapping peaks.

3) In general, but especially with overlapping components, it is necessary to use as much prior knowledge as possible in the quantification method to resolve the signal components. Therefore, it must be possible to incorporate the use of prior knowledge in the quantification method (20,21,22,23).

4) A choice must be made for a black box or a user interactive data processing method (24). The advantage of a black box method is that little or no involvement of the user is required. The advantage of user interactive methods is that they allow more operator intervention to optimize the fitting procedure. Therefore these methods can give better results.
In chapter 6 it is shown how a time domain data processing technique, in combination with the use of prior knowledge, can be used to analyze in-vivo MRS signals.

6 OUTLINE OF THIS THESIS

In chapter 2 a detailed description is given of several aspects concerning the use of multiple quantum coherence techniques for in-vivo studies.

In chapter 3 it is shown that a zero quantum sequence can be used to selectively measure lactate and that this sequence can be combined with a localization technique.

In chapter 4, editing techniques to separate signals from two complex spin systems (glutamate and glutamine) are described. Since it appeared to be very difficult to selectively measure one of these systems and suppress the other with multiple quantum coherence techniques, an alternative method is also given.

In cooperation with the Department of Experimental Medicine of the Academic Medical Center of the University of Amsterdam, pathophysiological mechanisms are studied which are responsible for the induction of hepatic encephalopathy (25,26). In the frame of this cooperation a study is performed in which an editing sequence is used to detect lactate, and a non-editing sequence to measure the complete spectrum. Results of this study are described in chapter 5.

Chapter 6 is concerned with data processing techniques. It is described how prior knowledge is incorporated in a Gauss-Newton iterative fitting procedure. Besides this, it is shown how actual prior knowledge can be obtained and used to analyze a signal consisting of damped sinusoids which in the frequency domain severely overlap.

Furthermore, the method is used to fit, frequency selectively, real in-vivo signals.

Concluding remarks and a summary are given in chapter 7.
REFERENCES

11) D.S. Stephenson, Prog. NMR Spectrosc. 20, 515 (1988).
26) I.R. Crossley, E.N. Wardle, R. Williams, Clinical Science n64, 247 (1983).
CHAPTER 2. (paper)

COMPARISON OF DOUBLE AND ZERO QUANTUM NMR EDITING TECHNIQUES

FOR IN-VIVO USE

J.E. van Dijk, A.F. Mehlkopf, W.M.M.J. Boveé*.

Submitted to NMR in Biomedicine.

RUNNING TITLE:
Comparison of in-vivo zero and double quantum techniques

* To whom correspondence should be addressed.

Department of Applied Physics, Section SST/SI, Delft University of Technology, P.O. Box 5046, 2600 GA Delft, The Netherlands.
ABSTRACT

Several multiple quantum editing techniques for in-vivo proton NMR are discussed and compared using simulated and experimental data. Extensions of these techniques for improved editing are given. Relative signal to noise ratios, modulation characteristics, metabolite selectivity, $B_0$ and $B_1$ inhomogeneity and motion effects are considered. Frequency selective read pulses can be used for signal enhancement and lipid suppression. Extra suppression of undesired signals can be obtained with a two shot $T_1$ cycle which gives good results for both the zero- and double quantum sequences. These sequences give at most 50% signal intensity and lipid suppression factors of about 2000 and 7000 respectively. A sequence which selects zero and double quantum coherences yields 100% signal intensity but only gives a good lipid suppression factor (7000) with phase cycling. It is shown that the multiple quantum modulation can be used to obtain specific metabolite editing. The effects of $B_0$ inhomogeneity on the multiple quantum coherences can be corrected for. $B_1$ inhomogeneity affects the investigated multiple quantum sequences in about the same way and decreases the volume of interest. In-vivo measurements show the good performance of the proposed zero- and double quantum sequences for lactate.

1. INTRODUCTION

For biomedical studies proton NMR Spectroscopy (MRS) is an important tool which makes it possible to measure relative concentrations in-vivo in a non-invasive way. The obtained proton spectra however contain many overlapping lines, with a large variation in peak intensity. This makes it difficult to quantitate the concentrations of metabolites accurately. Therefore, it is of great importance to develop techniques to edit the spectrum, so that only a certain range of metabolites is measured, e.g. the coupled or the uncoupled spin
systems. Further editing can be obtained by measuring signals from a certain order of multiple quantum coherences. In the extremest case only one metabolite is measured. Multiple quantum editing techniques for high resolution applications have been described elsewhere (1). In this work we compare several multiple quantum editing techniques concerning their advantages and disadvantages for in-vivo application, using simulated and experimental data. Because of the large variation in peak intensities, phase cycling schemes, used in-vivo, will give huge subtraction errors due to motion. For this reason we investigated sequences which select multiple quantum coherences with gradient pulses. Extensions to the multiple quantum techniques to obtain improved editing capabilities are given. For several implementations of zero and double quantum techniques, the relative signal to noise ratio of the detected signal, and the metabolite selectivity are investigated. The multiple quantum coherence modulation of the detected signal is fully exploited, leading to an improved selectivity. Furthermore, water and lipid suppression, which for in-vivo use is very important, as well as $B_0$ and $B_1$ inhomogeneity effects are considered. Although phase cycling methods are unfavorable for in-vivo studies, these methods are briefly discussed here. The resulting signal intensity and the multiple quantum modulation, which are important for the editing capabilities, differ from methods which use gradient selection. First a short overview of previously used editing techniques is given. After that, multiple quantum techniques will be discussed.

2. EDITING TECHNIQUES

Several editing techniques for in-vivo proton MRS have been proposed but only a few have been used for real in-vivo studies. It is possible to select signals from different compounds using differences in relaxation constants. This can be done with a spin echo experiment using long and short echo times. Other techniques are based on
differences between scalar coupling constants and differences in chemical shifts. Two basic methods can be distinguished. The first one is a two scan spin echo experiment, while in the second scan selective decoupling is used (2), or the 180 pulse is made frequency selective (3,4,5). The second method is based on homonuclear polarization transfer (6,7,8,9,10). The latter sequences, however, give a considerable loss of signal to noise ratio especially when for the lactate detection the $\alpha$-proton polarization is transferred to the CH$_3$ group. Brereton et al (11) proposed an editing method using three frequency selective 90° pulses to create correlated z-order magnetizations. This method yields 100% of the signal intensity but has two disadvantages. It does not differentiate between similar spin systems (e.g. lactate and alanine) and the authors state that, although this could be reduced, the sequence is sensitive to the frequency of their second selective pulse. Besides the methods mentioned above, multiple quantum techniques have proven to be useful to edit signals from certain spin systems. These techniques can use scalar coupling, chemical shift differences and additionally multiple quantum frequencies to obtain editing capabilities. The use of multiple quantum frequencies is especially of importance when two metabolites with similar and severely overlapping spectra (e.g. lactate and alanine) must be separated. The main disadvantage of multiple quantum techniques however is that in most cases a reduction of the signal to noise ratio occurs.

3. MULTIPLE QUANTUM COHERENCES

As mentioned before, one of the advantages of multiple quantum coherence (mqc) techniques is that an additional parameter, the modulation due to multiple quantum frequencies, can be used to distinguish different compounds by MRS. The distinction that can be achieved increases with the order of the mqc's used by the editing method. The obtained signal intensity however decreases with the order
d. 

\[ \text{Figure 1. Multiple quantum editing sequences. a) Double quantum sequence selecting the double quantum coherences with gradient pulses. All RF pulses are non-selective. b) Double quantum sequence as a), only the read pulse is frequency selective on the CH resonances (24). c) Multiple quantum sequence using phase cycling to select a certain order of coherences. d) Zero quantum sequence selecting the zero quantum coherences with a gradient pulse. e) Sequence proposed by Trimble et al (30), zero and double quantum coherences are selected with gradient pulses, } t = t_a + t_b. \text{ The selective } 180^\circ \text{ and } 90^\circ \text{ pulses excite only the CH resonances.} \]
of the mqc's. For this reason, and the low S/N ratio in in-vivo MRS, we decided to concentrate on zero and double quantum coherence techniques. Since we intend to use these techniques for in-vivo studies, it is of great importance to develop single, or at most two scan sequences to prevent motion artifacts as much as possible.

For AX₃ systems as lactate and alanine mqc's can be created with the first three pulses of the sequences shown in figure 1. Because of the 180° pulse the creation of the mqc's does not depend on the chemical shifts. This pulse also removes the effects of static field inhomogeneity. The mqc's evolve during τ₁ and are partly converted into antiphase magnetizations by the third 90° pulse. During τ₁ and τ₂ they evolve into detectable transverse magnetizations. If τ₁ = τ₂ the effects of the chemical shifts during τ₁ are compensated in τ₂ because of the last 180° pulse.

The effects of the sequences on a weakly coupled spin system can be calculated with the operator formalism as described by v.d. Ven et al (12) and Sorensen et al (13). The X spins are denoted by I₁, I₂ and I₃ and have frequencies in the rotating frame of ω₁, ω₂ and ω₃. The A spin is denoted by I₄ with frequency ω₄. Weak scalar coupling (coupling constant J) is assumed between the A and X spins. Signal intensities are given relative to the intensity of a FID signal.

\[ \sigma(t=0) = 2I_1 \frac{I_1 x}{4y} + 2I_1 \frac{I_2 x}{4y} + 2I_1 \frac{I_3 x}{4y} - 8I_1 \frac{I_1 I_2 I_3}{4x \cdot 3y \cdot 2y \cdot 1y} \]  

[1]

Effects of relaxation are omitted. At t=0 (see fig 1) the density matrix \( \sigma \) is given by [1] if \( \tau_0 = 1/(2J) \). This value for \( \tau_0 \) is used for all expressions given below.

In some studies (14,15,16) the optimal value of \( \tau_0 \) is found to be approximately 1/(4J). Theoretically the signal intensity is at that \( \tau_0 \) value a factor \( \frac{1}{2} \sqrt{2} \) lower. Due to the shorter creation time there is less signal loss caused by relaxation effects. The overall effect might be a (slightly) higher signal intensity.
Magnetizations of the last term of [1] contribute only to signals at the CH frequency which have a low intensity and give in the spectrum peaks close to the water resonance. Because the signal at the CH$_3$ frequency can be detected much easier, further on this last term will be omitted. The first three terms of [1], which finally give signals at the CH$_3$ frequency, behave identical. For this reason only the first term will be described. The index R (reduced) will be used on $\sigma$ to indicate that only coherences that contribute to the signal, detected during $t^2$, are analyzed. The double and zero quantum frequencies are $\omega = \omega_1 + \omega_4$ and $\omega = \omega_1 - \omega_4$, respectively.

To detect only signals corresponding to a certain order of mqc’s, all other coherences (including those of water and lipids) must be eliminated. This can be done with gradient pulses or with phase cycling schemes. Phase cycling methods are unfavorable for in-vivo studies because subtraction errors due to motion might be huge. Despite this, the resulting signal intensities and the multiple quantum modulation, which are important for the editing capabilities, are described here because they are not the same as with the gradient selection. Besides this, these differences are not yet described in literature. Another possibility of separating signals of different multiple quantum orders is described by Ernst et al (1). The RF frequency can be chosen such, that all multiple quantum frequencies of a certain order of multiple quantum coherences are within specific, not overlapping frequency ranges. This method however can only be used if a two dimensional data set is acquired and will not be considered here.

For in-vivo applications the mqc orders are most conveniently separated by $B_0$ gradients (17). The first term of [1] can be rewritten in terms of the upper- and lowering operators such that the zero and double quantum coherences can be distinguished:

$$2 \text{I}_1 \text{I}_4 = \frac{i}{2} (-\text{I}_1 \text{I}_4 + \text{I}_1 \text{I}_4) + \frac{i}{2} (-\text{I}_1 \text{I}_4 + \text{I}_1 \text{I}_4)$$
At \( t = t_0 + t_1^- \), just before the third 90° pulse, the density matrix is given by:

\[
\sigma_R(t_0 + t_1^-) = \frac{i}{2} \left\{ - I_+ I_4^+ \exp[i(\omega t_1 + 2G_1)] + I_1^- I_4^- \exp[-i(\omega t_1 + 2G_1)] \right. \\
+ I_1^+ I_4^- \exp[i\omega t_1] - I_1^- I_4^+ \exp[-i\omega t_1] \left. \right\} \cos^2(\pi Jt_1) \tag{2}
\]

with \( G_1 = \int_0^{t_{G1}} G_{1r}(t) \, dt \), \( r = x, y \) or \( z \).

The density matrix \( \sigma_R(t_0 + t_1^-) \) of [2] consists of double and zero quantum coherences. The double quantum term will be described in sections 3.1.1., 3.1.2. and 3.1.3. The zero quantum term will be described in 3.2.1. and 3.2.2.

3.1 DOUBLE QUANTUM COHERENCE SEQUENCES

Several groups have investigated double quantum pulse sequences for in-vivo studies. Knüttel et al (18) described a volume selective double quantum filtered sequence which yields 1/8 of the signal intensity. Nosel et al (19) presented simulations and phantom measurements using the same double quantum sequence as Dumoulin (20,21), concluding that the sequence is not optimal to measure lactate. Sotak et al (16) and Freeman et al (22) used double quantum, two dimensional sequences to measure lactate signals of implanted tumors in mice. Recently Crozier et al (23) presented some in-vivo two dimensional glutamate/glutamine and lactate edited spectra. Hurd et al (24) described a proton double quantum method which was used for in-vivo imaging of lactate. A disadvantage of two dimensional methods is the great sensitivity to motion artifacts.
3.1.1 SELECTION OF DOUBLE QUANTUM COHERENCES BY GRADIENT PULSES

In figure 1a, a 180° pulse is added at the end of the mqc sequence, to create an echo. If gradient pulses are used to select the double quantum coherences (dqc's) as in the sequence of figure 1a, during t$_1$ not only the zeeman and coupling hamiltonians have effect on the density matrix, but also gradient G$_1$ which is applied during t$_{G1}$. For the density matrix just before the third 90° pulse follows for the dqc terms from [2]:

\[
\sigma_R(t_0 + t_1^-) = \frac{i}{2} \left\{ -I_1^+ I_4^+ \exp[i(\omega t_1 + 2G_1 t_{G1})] \\
+ I_1^- I_4^- \exp[-i(\omega t_1 + 2G_1 t_{G1})] \right\} \cos^2(\pi J t_1)
\]  

[3]

The dqc's of the density matrix can also be described in terms of I$_x$ and I$_y$:

\[
\sigma_R(t_0 + t_1^-) = I_{1x} I_{4y} \cos(\omega t_1 + 2G_1) \cos^2(\pi J t_1) \\
+ I_{1x} I_{4x} \sin(\omega t_1 + 2G_1) \cos^2(\pi J t_1) \\
- I_{1y} I_{4y} \sin(\omega t_1 + 2G_1) \cos^2(\pi J t_1) \\
+ I_{1y} I_{4x} \cos(\omega t_1 + 2G_1) \cos^2(\pi J t_1)
\]  

[4]

At the end of t$_1$ the read pulse converts the first term into antiphase magnetizations, the other terms of [4] do not contribute to the detected $X_3$ signal. The antiphase magnetizations evolve during $\tau_1$ and $\tau_2$ into detectable magnetizations. If $\tau_1 = \tau_2$ the effect of the zeeman hamiltonian during $\tau_1$ is compensated in $\tau_2$ because of the second 180°
pulse. This means that only the coupling and the gradient effects have to be calculated:

\[
\sigma_R(t = 0) = \frac{1}{4} \sin(\pi J(\tau_1 + \tau_2)) \cos^2(\pi J t_1)
\]

\[
= \left( I_{1y} \begin{bmatrix} \cos(\omega D_1 t_1) [\cos \alpha + \cos \beta] - \sin(\omega D_1 t_1) [\sin \alpha + \sin \beta] \\ \sin(\omega D_1 t_1) [\cos \alpha - \cos \beta] + \cos(\omega D_1 t_1) [\sin \alpha - \sin \beta] \end{bmatrix} + I_{1x} \begin{bmatrix} \sin(\omega D_1 t_1) [\cos \alpha + \cos \beta] - \sin(\omega D_1 t_1) [\sin \alpha + \sin \beta] \\ \sin(\omega D_1 t_1) [\cos \alpha - \cos \beta] + \cos(\omega D_1 t_1) [\sin \alpha - \sin \beta] \end{bmatrix} \right)
\]

[5]

with \( \alpha = 2 G_1 + G_2 \), \( \beta = 2 G_1 - G_2 \).

If the gradients are chosen according to \( 2G_1 = G_2 \) (\( \beta = 0 \)), half of the dqc's (anti echo) are refocused by the second gradient. The density matrix at \( t_2 = 0 \) is then described by:

\[
\sigma_R(t_2 = 0) = \frac{1}{4} \cos^2(\pi J t_1) \sin(\pi J(\tau_1 + \tau_2)) \left\{ I_{1y} \cos(\omega D_1 t_1) - I_{1x} \sin(\omega D_1 t_1) \right\}
\]

[6]

Note that the effective double quantum modulation frequency is \( -\omega_D \).

If \( 2G_1 = - G_2 \) (\( \alpha = 0 \)) the other part of the double quantum coherences are refocused (echo) and

\[
\sigma_R(t_2 = 0) = \frac{1}{4} \cos^2(\pi J t_1) \sin(\pi J(\tau_1 + \tau_2)) \left\{ I_{1y} \cos(\omega D_1 t_1) + I_{1x} \sin(\omega D_1 t_1) \right\}
\]

[7]

Now the double quantum modulation frequency is \( +\omega_D \).

The results given in [6] and [7] show that the signal measured during \( t_2 \) is phase modulated with \( \pm \omega D_1 \). The signal intensity is only 1/4 of the total magnetization of the observed spins. This can be understood
since half of the magnetization is at $t=t_0$ converted into zqc's and half into dqc's. Because of the gradient pulses, signal is detected from only the echo or the anti echo which again halves the signal intensity.

An advantage of applying the second gradient pulse after and not before the $180^\circ$ pulse, is that unwanted magnetizations resulting from this pulse are destroyed. A disadvantage is more risk for signal loss due to motion artifacts because the time between $G_1$ and $G_2$ is larger. The best solution is to spoil gradient pulses before and after the last $180^\circ$ pulse and applying $G_1$ and $G_2$ immediately before and after the read pulse.

After editing by selecting a certain mqc order, further editing is possible by using the mqc frequencies to separate (nearly) overlapping signals, e.g. the CH$_3$ groups of alanine and lactate, see section 8. This can be done with a two scan measurement using two values for $t_1$, $t_{11}$ and $t_{12}$ respectively, such that

$$(\omega_0)_{1ac} (t_{11} - t_{12}) = 2 \pi k_1$$

and

$$(\omega_0)_{ala} (t_{11} - t_{12}) = (2k_2 + 1) \pi k_1, k_2 \in \mathbb{N}.$$  \[8\]

The lactate signal is two times measured with the same sign while the alanine signal is inverted in the second measurement. By adding or subtracting, spectra are obtained with respectively only lactate or alanine doublets. If the difference $t_{11} - t_{12}$ is not much smaller than $1/J$, a correction for the modulation due to coupling effects is necessary.

It is also possible to modulate the lactate CH$_3$ group through an odd number of half cycles of the double quantum modulation frequency during the period $t_{11} - t_{12}$. In that case the alanine CH$_3$ group should modulate an even number of half cycles.

Further editing can also be achieved with a two scan measurement using an inverted gradient ($G_1$ or $G_2$) in the second scan. In this case the $I_{1x} \sin(\omega t_{11})$ terms of [6] and [7] cancel after addition of the
signals. The result is a signal which is amplitude modulated with $\omega D^t_1$. If $t_1$ is chosen such that

$$(\omega D)^{t_1}_{asc} = k_1 \pi$$

and

$$(\omega D)^{t_1}_{ala} = (2k_2 + 1) \frac{\pi}{2} \quad k_1, k_2 \in \mathbb{N}.$$  \[9\]

The two measurements yield a spectrum with the lactate doublet but without the alanine doublet. The latter method is sensitive to eddy currents. This can be circumvented by optimizing $G_2$ in both scans such that the effects of the eddy currents are compensated.

3.1.2. DOUBLE QUANTUM SIGNAL ENHANCEMENT BY A SELECTIVE READ PULSE

Several authors (24,25,26) have already described that the intensity of the double quantum signal can be increased. This is done by using a frequency selective pulse (on the A spin) to convert the dqc's into antiphase magnetizations, see the pulse sequence of figure 1b. This selective read pulse not only converts the first term of [4] but also the third term into antiphase magnetization. Both terms equally contribute to the signal intensity measured during $t_2$.

With $\tau_1 = \tau_2 = (2k+1)/(4J) \in \mathbb{N}$ and $2G_1 = G_2$ the density matrix at $t_2=0$ is given by

$$\sigma_R(t=0) = \frac{1}{2} \cos^2(\pi J t_1) \left\{ I_{1y} \cos(\omega D t_1) - I_{1x} \sin(\omega D t_1) \right\}$$ \[10\]

If however the gradients are chosen such that $2G_1 = - G_2$, no signal will be detected during $t_2$. This is because the contributions of $I_{1x} I_{4y}$ and $I_{1y} I_{4y}$ of [4] to the detected signal cancel. Of course, if the second gradient is applied before the last 180° pulse, this gradient must have an opposite sign. The signal measured during $t_2$ is
phase modulated with $-\omega_{D_1} t_1$. Since there is no signal if the gradients have opposite signs, the only possibility to separate overlapping CH$_3$ doublets is to use two values for $t_1$ as in [8]. From [10] is clearly seen that the signal intensity is doubled as compared to [6] or [7]. The experimental verification is described in section 8.

3.1.3 SELECTION OF DOUBLE QUANTUM COHERENCES BY PHASE CYCLING

The dqc contributions can also be selected by phase cycling. Using the pulse sequence of figure 1c, ($\phi = x, y, -x, -y$; acquisition: $+, -, +, -$) the density matrix, divided by the number of scans of the phase cycle, is given by [11] if again $\tau_1 = \tau_2 = (2k+1)/(4J)$ with $k \in \mathbb{N}$.

$$\sigma_{R}(t_2=0) = \frac{1}{2} \sum_{y} \cos(\omega_{D_1} t_1) \cos^2(\pi J t_1)$$

Expression [11] shows that the maximal double quantum signal intensity is obtained as in the previous section. The signal measured during $t_2$ is amplitude modulated with $\omega_{D_1} t_1$. This means that as in section 3.1.1 e.g. lactate or alanine can be measured selectively in a single scan. If $t_1$ is chosen according to [9] a spectrum can be obtained with the lactate but without the alanine doublet.

3.2 ZERO QUANTUM SEQUENCES

Doddrell et al (27) used a single shot, zero quantum sequence with frequency selective pulses, to measure brain extracts. Their sequence, which yielded 1/4 of the maximal CH$_3$ signal intensity of lactate, contained a 180° pulse in the middle of the multiple quantum evolution period. With this pulse the zero quantum coherences are rephased. Gradient pulses are used to prevent refocusing of non-zero quantum coherences. The multiple quantum evolution period had to be 1/J. This long evolution period and the 75 % loss of signal intensity makes the
sequence undesirable for in-vivo use. Sotak et al (28) used a zero quantum, two dimensional sequence to measure lactate. A disadvantage of the two dimensional method is the great sensitivity to motion artifacts. Sotak et al (29) also described a localized stimulated echo zero quantum sequence which was used to measure, postmortem, lactate signals from the rabbit kidney. With this zero quantum method lactate was edited by subtraction of two spectra. The loss of signal intensity is 50 % if all experimental parameters are optimal. The creation of zero quantum coherences with their sequence depends on the single quantum frequencies. This can be circumvented by using a 180° pulse during the creation period as described before.

In sections 3.1.1, 3.1.2 and 3.1.3 only the double quantum term of [2] was described. Now the zero quantum coherence (zqc) term, given in [12] will be discussed.

\[ \sigma_{Rz_{0}} \tau_{1}^{+}= \frac{1}{2} \left\{ I_{1+} I_{1-} \exp[i\omega_{z}t_{1}] + I_{1-} I_{1+} \exp[-i\omega_{z}t_{1}] \right\} \cos^{2}(\pi Jt_{1}) \]  

[12]

### 3.2.1 SELECTION OF ZERO QUANTUM COHERENCES BY A GRADIENT PULSE

With the sequence of figure 1d, during \( t_{1} \) all coherences are dephased by gradient pulse \( G_{1} \). Only the zqc's and longitudinal magnetizations are not affected by this pulse. To detect transverse magnetizations during \( t_{2} \), resulting from the zqc's, the gradient pulse \( G_{2} \) during \( \tau_{1} \) or \( \tau_{2} \) as in sequence 1b, must not be applied. Suppression of the longitudinal magnetizations (present during \( t_{1} \)) is discussed later in this section. The density matrix at \( t_{2}=0 \) is given by \( \tau_{1} = \tau_{2} = (2k+1)/(4J) \) with \( k \in \mathbb{N} \).

\[ \sigma_{Rz_{2}}(t=0) = \frac{1}{2} I_{1y} \cos^{2}(\pi Jt_{1}) \cos(\omega_{z}t_{1}) \]  

[13]

From this expression can be seen that the maximal zero quantum signal
is detected in a single scan measurement, which however still results in 50% loss of signal intensity. Furthermore, the measured signal is amplitude modulated. This makes it possible to selectively detect again e.g. alanine or lactate as in 3.1.1 and 3.1.2 in a single scan. If $t_1$ is chosen such that

$$\omega_{z,1ac} t_1 = k_1 \pi$$

and

$$\omega_{z,ala} t_1 = (2k_2 +1) \frac{\pi}{2}$$

$k_1, k_2 \in \mathbb{N}. \quad [14]$ 

the lactate doublet signal is measured and the alanine doublet signal is suppressed.

A disadvantage of the zero quantum pulse sequence of figure 1d is that non-coupled spins also contribute to the detected signal. During $t_1$ the magnetization vector of these spins is aligned along the z axis and is therefore insensitive to the gradient pulse. After applying the last two RF pulses, their echo signal is fully obtained since a second gradient pulse is not applied as in the dqc sequences. To suppress these signals, a two scan zero quantum measurement with two values for $t_1$ can be used (29). The difference in $t_1$ is $\Delta t_1 = k \pi / (\omega_z^1)$, $k \in \mathbb{N}$. The signals of the non-coupled spins have the same phase but the phase of the lactate (or alanine) signal differs $180^\circ$ in the two measurements as can be seen from [12] or [13]. In the difference of the two signals, contributions of the non-coupled spins are eliminated. Because $\Delta t_1$ can be very small, on our 7 Tesla system $\Delta t_1 = 0.6$ msec (k=1), the effects of relaxation and the change in $\cos^2(\pi Jt_1)$ can be ignored.

Ernst et al (1) described that zqc's are insensitive to the phases of the creation pulses. The single quantum coherences however, are sensitive to these phases. This difference in sensitivity can be used instead of the $t_1$ cycle. If in the first scan the creation pulses have phases $\phi$ and in the second scan $\phi + 180^\circ$ the zero quantum signals have the same phase in the two measurements, but the single quantum coherences are $180^\circ$ out of phase in the second measurement. An
advantage of this method is that there are no effects due to relaxation or the change in $\cos^2(\pi Jt_1)$. A disadvantage however is that lipid zqc signals are not suppressed as with the previously described $t_1$ cycle, see section 4. Note that if phase cycling is used to select zero quantum coherences (see section 3.2.2), huge signals must be subtracted which gives rise to subtraction errors. If the effects due to relaxation and coupling during $\Delta t_1$ can be ignored, the $t_1$ cycle, in combination with other water and lipid suppression methods, will only give errors which are equal to those of normal signal averaging. Therefore, instead of averaging which in-vivo is always necessary because of low signal to noise ratios, $t_1$ cycling can be applied with the zero and double quantum sequences.

It is obvious that the methods described before are not able to sufficiently suppress huge water and lipid signals. The in-vivo water and lipid suppression will be discussed in section 4.

3.2.2 SELECTION OF ZERO QUANTUM COHERENCES BY PHASE CYCLING

If the pulse sequence of figure 1c is used ($\phi = x, y, -x, -y$ acquisition $+++-$) only the zero quantum coherences contribute to the signal measured during $t_2$. The density matrix at $t_2 = 0$, divided by the number of scans of the phase cycle, is given in [13]. So the $t_1$ modulation is the same when selecting zqc’s by gradient pulses or by phase cycling. The signal contributions of non-coupled spins however are eliminated by the phase cycling method. For the water signal, additional suppression methods are necessary in-vivo, see section 4.

3.3. COMBINED ZERO AND DOUBLE QUANTUM COHERENCES.

Trimble et al (30) recently described how the detected signal intensity can be further improved. Signals corresponding to the zero
and the double quantum coherences are measured simultaneously during $t_2$. This is done with the sequence of figure 1e. During $t_1 = t_a + t_b$, the first gradient pulse dephases the dqc's. A selective $180^\circ$ pulse (on the A spin) in the middle of $t_1$ converts the zqc's in dqc's and the dqc's in zqc's. It is also possible to use a selective pulse on the X spins. The second gradient pulse dephases the dqc's then present. At the end of $t_1$ the zero and the double quantum coherences present at $t = 0$ are dephased to the same extent. After the selective read pulse, which has the effect as described in section 3.1.2, the magnetizations resulting from both the zero and the double quantum coherences are rephased by the last gradient pulse. The density matrix at $t_2 = 0$ is given by $(2G_1 = 2G_2 = G_3$ and $\tau_1 = \tau_2 = (2k+1)/(4J)$):

$$
\sigma_{R_2}(t = 0) = -\frac{1}{2}I_{1y} \left\{ \cos(\omega_1 t_a + \omega Z_b) + \cos(\omega Z_a + \omega D b) \right\}
+ \frac{1}{2}I_{1x} \left\{ \sin(\omega_1 t_a + \omega Z_b) + \sin(\omega Z_a + \omega D b) \right\}
$$

[15]

The signal measured during $t_2$ is neither amplitude-, nor purely phase modulated. If $t_a = t_b$ and $\omega = 0$ the zero and double quantum frequencies are equal and the signal is phase modulated with $2\omega_1 t_a$. Note that the signal intensity is maximal (100 %), which is very important for in-vivo studies, see also section 8. Because of the selective $180^\circ$ pulse the coupling during $t_1$ does not modulate the signal. As described, gradient pulses are used to select the double and the zero quantum coherences, and a frequency selective read pulse is used to convert the mqc's into antiphase magnetizations. Additionally Trimble et al used a phase cycling scheme to suppress signals from unwanted coherences, see table 1. A disadvantage of this sequence is that at least three gradient pulses must be applied. These pulses are difficult to adjust, especially on systems suffering from eddy currents.
Table 1. Phase cycling scheme proposed by Trimble et al (30) for the sequence of figure 1e.

<table>
<thead>
<tr>
<th>step</th>
<th>$\phi$</th>
<th>$\psi$</th>
<th>$\theta$</th>
<th>receiver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$+x$</td>
<td>$+x$</td>
<td>$+y$</td>
<td>$+$</td>
</tr>
<tr>
<td>2</td>
<td>$+x$</td>
<td>$+y$</td>
<td>$+y$</td>
<td>$-$</td>
</tr>
<tr>
<td>3</td>
<td>$+x$</td>
<td>$-x$</td>
<td>$+y$</td>
<td>$+$</td>
</tr>
<tr>
<td>4</td>
<td>$+x$</td>
<td>$-y$</td>
<td>$+y$</td>
<td>$-$</td>
</tr>
<tr>
<td>5</td>
<td>$-x$</td>
<td>$+x$</td>
<td>$+y$</td>
<td>$+$</td>
</tr>
<tr>
<td>6</td>
<td>$-x$</td>
<td>$+y$</td>
<td>$+y$</td>
<td>$-$</td>
</tr>
<tr>
<td>7</td>
<td>$-x$</td>
<td>$-x$</td>
<td>$+y$</td>
<td>$+$</td>
</tr>
<tr>
<td>8</td>
<td>$-x$</td>
<td>$-y$</td>
<td>$+y$</td>
<td>$-$</td>
</tr>
</tbody>
</table>

4. SUPPRESSION OF UNWANTED RESONANCES

The water and lipid suppression, which is essential for in-vivo studies, is for the double quantum measurements relatively simple if gradient pulses are used to select the double quantum coherences. In that case, gradient $G_2$ (figure 1a, 1b) dephases the unwanted magnetizations resulting from uncoupled protons. Contributions from coupled lipid protons can be eliminated with the selective read pulse (24). This can easily be understood if it is realized that the coupled protons of the lipids are not affected by the selective pulse. This means that the multiple quantum coherences are not converted into transverse magnetizations. If phase cycling is used to select double or zero quantum coherences, the unwanted signals must be eliminated by additions and subtractions. For the intense water and lipid signals this is nearly impossible because of the high requirements which in that case are needed for e.g. the dynamic range of the receiver, the system stability and the immobility of the animal.
The zero quantum pulse sequence, including the $t_1$ cycle, does not efficiently suppress the signals from uncoupled spins. Huge water and lipid signals for instance, can not be eliminated in-vivo by subtraction techniques. In order to suppress the water signal, we use a frequency selective adiabatic pulse to invert the water resonances. The sequence starts at a time $t_0$ after this pulse; $t_0$ is chosen to minimize the remaining water signal (31). This method can also be used as an additional water suppression technique for the double quantum pulse sequences.

The lipid signal arising from uncoupled spins can be eliminated by the $t_1$ cycle in combination with a frequency selective read pulse on the $A$ spins of the $AX_2$ system (32). After such a pulse the undesired magnetization vectors remain longitudinal, and therefore do not contribute to the detected signal. If at the end of the sequence, a $180^\circ$ pulse is used to obtain an echo, water and lipid signals, which might be created due to pulse imperfections, can be destroyed with spoil gradients. Till now, only uncoupled water and lipid protons have been discussed. Coupled lipid protons however, can give rise to zqc's which contribute to the detected signal. With the $t_1$ cycle and the selective read pulse these contributions are suppressed. In the following we describe a worst case calculation: From FID spectra of animal fat and bone marrow it appeared that in the range $1.3 \pm 0.2$ ppm, zqc signals can be expected from the following lipid protons (marked with an asterisk):

$$0 = \text{C} - \text{CH}_2 - \text{CH}_2^*, \quad \text{CH}_2^* - \text{CH}_2 - \text{CH} = \text{CH} - \text{CH}_2 - \text{CH}_2^*, \quad \text{CH}_2^* - \text{CH}_3.$$ 

The zero quantum frequencies are 0.28, 0.71, 0.71 and 0.40 ppm respectively. Assuming a 1:1 ratio of zero and double quantum contributions of the eight marked protons, the total zero quantum intensity of these signals is at most 12% of the normal single quantum lipid CH$_2$ peak. Taking into account the different zqc frequencies, it can be calculated that the $t_1$ phase cycle and the frequency selective

31
read pulse (133I) leave at most 0.2% of the total lipid signal. This means that in theory a lipid suppression of 500 can be achieved. However, it is assumed that the zqc creation is optimal. Since this is not completely true, in measurements a higher lipid suppression can be expected. Experimental results, with indeed a lipid suppression of more than 2000, are described in section 8.

In theory, using the sequence of figure 1e, unwanted signals are destroyed by G3 and by the selective read pulse. Additionally Trimble et al (30) used a phase cycling scheme to suppress signals from single quantum coherences. We found that this phase cycling scheme is of great importance to obtain a good lipid suppression, see section 8 and table 2.

5. B0 FIELD INHOMOGENEITY EFFECTS

For single quantum measurements a poor homogeneity results in broadened lines. Double quantum coherences are twice as sensitive to B0 field inhomogeneity as single quantum coherences. If a certain spin system has single quantum frequencies \( \omega_{1} + \delta(r) \) and \( \omega_{4} + \delta(r) \), the double quantum frequency is given by \( \omega_d + 2\delta(r) \). The inhomogeneity component \( \delta(r) \) in rad/s depends on the location of the spin system in the static magnetic field. Because the double quantum coherences are detected after they are converted into single quantum coherences, the same line broadening results as in a single quantum spectrum. Besides this however, there is a line distortion resulting from the B0 inhomogeneity which affects the double quantum modulation. As an example, the effects of this distortion will be explained for the two shot measurement with the alternating gradient pulses as described in paragraph 3.1.1. The signal measured during \( t_2 \) is amplitude modulated with \( \cos(\omega_D t_1) \). If however an inhomogeneity term \( \delta(r) \) (rad/s) is assumed, the modulation is given by \( \cos(\omega_D t_1 + 2\delta(r)t_1) \). In practice the choice for \( t_1 \) is such that \( \cos(\omega_D t_1) = 1.0 \) in order to observe maximal signal intensity. This means that \( \sin(\omega_D t_1) = 0.0 \) and
therefore

\[ \cos(\omega \, t_1 + 2\delta(r) \, t_1) = \cos(\omega \, t_1)\cos(2\delta(r) \, t_1) - \sin(\omega \, t_1)\sin(2\delta(r) \, t_1) \]

\[ = \cos(2\delta(r) \, t_1) \]

[16]

Figure 2. Spectrum of the alanine CH₃ resonance of a 50 mM alanine solution in H₂O measured from a 5 mm NMR tube. Poor homogeneity was obtained by a missetting of the B₀ correction shims. --- --- : 90-180 spin echo sequence with a binomial 180 pulse (11) to suppress the water resonances, echo time 136 ms. --- : spectrum obtained with the double quantum sequence of figure 1a with gradient pulses as described in section 5. x x x : lineshape distortion calculated with eq. [16]. The spectra are normalized.
Eq [16] shows that the resonance line is attenuated with \( \cos(2\delta(r)t_1) \) because of \( B_0 \) inhomogeneity. The effects of the distortion can be seen in figure 2. A poor homogeneity was obtained by missetting the \( B_0 \) correction shims. At the top of the peak \( \delta(r)=0 \) and \( \cos(2\delta(r)t_1)=1 \), therefore the height of the peak is not affected by this distortion.

At a frequency \( \delta \) beside the top, the peak height is attenuated by a factor \( \cos(2\delta t_1) \). If \( \tau_1 \) and \( \tau_2 \) are chosen according to \( 2\tau_1 + \tau_1 = \tau_2 \) the inhomogeneity effects of the double quantum coherences are compensated in \( \tau_2 \). Of course, to measure the doublet in phase and not (partially) in antiphase, the restriction \( \tau_1 + \tau_2 = (2k+1)/(2J) \), \( k \in \mathbb{N} \) should be fulfilled.

The same type of lineshape distortion is obtained when data acquisition of the dqc echo is initiated prior to the top of the echo, even when the sample is well shimmed. In fact, the \( B_0 \) inhomogeneity affects the point in time at which the dqc echo refocuses because the dqc dephasing during \( t_1 \) adds to the dephasing during \( \tau_1 \). By shifting the 180° pulse as described before, this effect is eliminated.

In conclusion, for the described example it is possible to correct for the \( B_0 \) inhomogeneity, resulting in an increased integrated signal intensity. For other applications of mqc editing techniques, the influence of this inhomogeneity must be analyzed to prevent signal loss.

6. \( B_1 \) FIELD INHOMOGENEITY EFFECTS.

If surface coils are used for excitation and detection, not only \( B_0 \) inhomogeneity but also \( B_1 \) inhomogeneity effects degrade the detected signal. Dumoulin (20) investigated how the creation of single- and multiple quantum coherences depends on the flip angle of the first two 90° pulses. Since \( B_1 \) is directly related to the applied pulse angle of all pulses, we investigated the detected signal intensity as a function of all rotation angles. This was done with a simulation program written and given to us by F.J.M. van de Ven, Department of
Figure 3. Signal intensity of some multiple quantum sequences of figure 1 as a function of the RF amplitude. The lines connect simulated points. The measured points are given by the asterisks. A cyclops and a half exor cycle of the last 180° pulse are used. Along the x axis the real flip angles of the "90°" pulses are given, the "180°" pulses are twice this value. For the frequency selective pulses the 1331 or 1331 binomial sequences were used. The measurements are normalized to the simulated value at 90°. All time intervals are given in msec. a) double quantum sequence of fig 1b, τ₀=68, τ₁=10, τ₂=24, τ₂=44, τ₁=6, τ₂=12, G=1 G=10 mT/m (z direction). Spoil gradients (x direction) before and after the first 180° pulse. b) zero quantum sequence of fig 1d, τ₀=68, τ₁=10, τ₂=34, τ₁=6, G=10 mT/m (z direction). Spoil gradients (x direction) before and after the 180° pulses. c) sequence of fig 1e without the phase cycle of table 1, τ₀=68, τ₁=10, τ₂=24, τ₂=44, τ₂=6, τ₂=6, τ₂=12, G=1 G=1 G=1 G=1 mT/m (z direction). Spoil gradients (x and y direction) before and after the non selective 180° pulses. d) Spin echo sequence with an exor cycled binomial (22) 180° pulse. The echo time is 136, the interpulse time of the 22 sequence is 0.6, spoil gradients before and after the 22 sequence are 20 mT/m during 4 ms.
Biophysical Chemistry, University of Nijmegen, The Netherlands. With this program the effects of pulse-, zeeman- and coupling hamiltonians on spin systems can be simulated. We incorporated gradient pulses. In figure 3 experimental and simulated data are shown for the sequences of figure 1b, d and e. From this figure it can be concluded that the simulated and the experimental data are consistent and that the three sequences have the same $B_1$ dependence. Because of the extra selective pulse in the middle of $t_1$, we expected the sequence of figure 1e to be more sensitive to $B_1$ inhomogeneity than the sequences of figure 1b and d. Obviously, $B_1$ inhomogeneity effects of the creation and read pulses determine the curves of figure 3. For comparison the same study is performed for a spin echo sequence with a binomial 180° pulse to suppress the water signal (figure 3d). We conclude that mqc techniques in combination with surface coils give an, usually undesired, extra localization effect. This means that due to the RF inhomogeneity a lower S/N ratio is obtained. To estimate this effect we calculated the flip angles in 1600 positions in a slice of 3 mm thickness parallel to the coil (diameter 12 mm), located at a distance of 2 mm from the coil. With these flip angles and the results presented in figure 3b and 3d we found that the zqc sequence yields only 34 % of the signal intensity as compared to the spin echo sequence.

7. LOCALIZATION COMBINED WITH MULTIPLE QUANTUM SEQUENCES

There are two methods to combine multiple quantum techniques with localization:

1) The 90° pulses of the sequences are made slice selective (18,28,29). This method has two important disadvantages. The first is that the read pulse can not be frequency selective anymore which results in a lower signal intensity for the dqc sequences and a lower lipid suppression for all multiple quantum sequences. The second disadvantage is that, if three slice selective 90° pulses are used, the mqc filter is only effective in a small volume. This
means that the suppression of the undesired signals from outside the volume of interest is poor.

2) Before or after the mqc sequence, additional localization pulses or phase encoding gradients are applied (9,21,23,33). This method does not have the disadvantages described before but the sequence is longer which might result in a lower signal to noise ratio due to relaxation.

For both methods it is important to note that gradient pulses, used for localization, might interfere with gradient pulses used for the selection of mqc's. If possible, orthogonal gradient directions must be used for these purposes.

Recently we showed (34) how localization can be achieved with adiabatic slice selective 360° pulses, which are incorporated in the sequence just before the last 180° pulse. This technique is based on method 2) and can be used with all multiple quantum sequences. The 360° pulse consists of two 180° pulses. The phase change within the volume of interest due to the first pulse is compensated by the second pulse (35). The adiabatic pulses are insensitive to $B_1$ inhomogeneity, and therefore, signal reduction due to $B_1$ inhomogeneity effects of these pulses will not occur.

8. EXPERIMENTAL RESULTS

All measurements described below are performed on a home built spectrometer (36) and a 7T Oxford magnet with a vertical 89 mm bore. To test the sequences, all measurements are performed with the $B_0$ homogeneity set to correspond to in-vivo linewidths.

To show the extended editing capabilities of the mqc sequences described in section 3, as an example we use the double quantum sequence of figure 1b, executed with two $t_1$ values as defined in equation [8]. The results of the two measurements on a 5 mm NMR tube containing alanine and lactate solved in water, are shown in figure 4. In spectrum b the alanine CH$_3$ resonances are 180° degrees out of phase.
Figure 4. Double quantum editing measurement on a 5 mm tube containing ± 15 mM lactate and alanine solved in H2O, only the CH3 resonances are shown. The sequence of figure 1b is used. The transmitter was positioned between the CH resonances of lactate and alanine. The t1 values correspond with k1=6 and k2=6 of Eq [8]. Gaussian windowing (21 ms) was applied as we usually do with in-vivo signals. Time intervals are given in ms: T0=68, T1=24, T2=44, t1=6, t2=12, G1=G2=10 mT/m (z direction). a) t1=24.6, b) t1=16.6, c) subtraction of a and b, the alanine spectrum, d) addition of a and b, the lactate spectrum.
as compared to spectrum a. The lactate doublet has the same phase in
the two spectra. The editing results after subtraction and addition
can be seen in spectra c and d.
We also used a 5 mm tube containing ± 20 mM alanine to verify the
relative signal intensities of the sequences b, d and e. Sequences a
and c of figure 1 are not used because 1a gives a low signal intensity
and 1c will give huge subtraction errors in in-vivo studies. For the
frequency selective pulses the 1331 or 133I sequences were used.
Sequence e was tested with and without the phase cycling scheme of

Table 2. Relative signal intensity and lipid suppression of the
multiple quantum sequences of figure 1b, 1d, and 1e.

<table>
<thead>
<tr>
<th>multiple quantum sequence</th>
<th>alanine peak area (%)</th>
<th>lipid CH&lt;sub&gt;2&lt;/sub&gt; suppression</th>
<th>number of scans</th>
</tr>
</thead>
<tbody>
<tr>
<td>double (fig 1b)</td>
<td>52 ± 10</td>
<td>6900 ± 100</td>
<td>16</td>
</tr>
<tr>
<td>zero (fig 1d)</td>
<td>58 ± 10</td>
<td>2100 ± 100</td>
<td>16</td>
</tr>
<tr>
<td>combined (fig 1e)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>without phase cycle</td>
<td>98 ± 10</td>
<td>60 ± 5</td>
<td>16</td>
</tr>
<tr>
<td>with phase cycle</td>
<td>100 ± 10</td>
<td>7000 ± 100</td>
<td>16</td>
</tr>
</tbody>
</table>

table 1. From the spectra, the peak area of the alanine CH<sub>3</sub> resonances
was calculated, see table 2. Sequence 1e indeed does give double
signal intensity. In figure 5 spectra are shown of the same sequences
applied on a phantom containing pure olive oil. In figure 6 the
spectrum of a FID, measured from this phantom is shown. The obtained
lipid suppression factor of the four sequences is given in table 2.
This factor is defined as the quotient of the peak areas of the peak
at 1.3 ppm of the FID and the multiple quantum spectrum. The double
quantum sequence gives the best suppression; the lipid suppression of

40
sequence 1e is very good if phase cycling is used but is very poor otherwise. All measurements have been performed with the $t_1$ cycle described in 3.1.1. We found that, if AM modulated frequency selective pulses are used instead of binomial pulses, lipid suppression factors higher than 10000 can be obtained with the zero and the double quantum sequences of figure 1b and 1d. These pulses were not used for all measurements presented here, because they are relatively long,

Figure 5. Measurement on a 5 mm tube containing pure olive oil. Time intervals are the same as in the legend to figure 3. a) dqc sequence of figure 1b, b) zqc sequence of figure 1d, c) the sequence of figure 1e without and d) with the phase cycling scheme of table 1. All spectra are obtained with 16 scans, including the $t_1$ cycling scheme. The dqc sequence gives the best suppression of the lipid peaks at 1.3 ppm; the sequence of figure 1e only gives a good suppression with the phase cycle.
Figure 6. FID with cyclosp phase cycling measurement of pure olive oil. The flip angle is 90°, repetition time is 4 sec. A scaling factor 800 compared to figure 5 is used.

resulting in long mqc evolution times which are undesirable. Because the lipid suppression of sequence 1e is poor without the phase cycling scheme, and because it is difficult to optimize the gradient pulses that select the zero and double quantum coherences, we do not use this sequence in-vivo. The sequences of figure 1b and 1d are now used on a routine basis. Below some results are presented as an example of an in-vivo application of mqc techniques. In the frame of our study of Hepatic Encephalopathy in rats, the effects of hyper ammonemia have been investigated. Two days before the experiments, an ellipsoidal surface coil (axes 13 and 10 mm) was implanted on the skull (37). Hyper ammonemia in male Wistar rats was
Figure 7. a) double and b) zero quantum in-vivo spectra of the brain of a rat, 10 minutes after infusion of ammonium acetate, see text. Time intervals are set as described in the legend to fig 3. The repetition time is 4 s, 48 scans for each spectrum were accumulated.

induced by intra-peritoneal administration of 7 mMol ammonium acetate per kg (38). The brain lactate concentration changes in time during hyper ammonemia and was measured every 15 minutes with the two mqc sequences. Both sequences were executed alternately and this combined sequence was repeated for signal averaging. In this way both sequences detected the lactate concentration in approximately the same time interval. The total measuring time of the combined sequence was 7 minutes. In figure 7 some typical results are shown. The lactate concentration is about 4 mM. In figure 8 an unedited spectrum is shown, measured with a water suppressed spin echo sequence, the lipid peak (1H concentration is about 20 mM) comes from two bone ridges on the skull. This peak is suppressed in the zero- and double quantum spectra. The latter is concluded because in zero- and double quantum
spectra measured before hyper ammonemia was induced no peaks in the region between 1 and 2 ppm were present. In some cases we found that the lactate intensity in the zero quantum spectrum is higher than in the double quantum spectrum. This can be explained by a slight missetting of the double quantum selection gradient pulses. In-vivo it is difficult to optimize these pulses. Because the dqc's are very sensitive for such a missetting, loss of signal intensity might occur.

Figure 8. Water suppressed in-vivo spectrum of the rat brain measured with a spin echo sequence with a binomial \((2^2)\) 180° pulse. Experimental conditions are given in the legend to figure 7. The echo time is 136 ms, the repetition time 4 sec, 40 scans including an excitation cycle of the 180° pulse, are accumulated.

9. COMPARISON OF THE TECHNIQUES AND CONCLUSIONS

Several multiple quantum techniques have been described in literature to edit in-vivo spectra. Since the detected signal intensity decreases with the order of the multiple quantum coherences from which signal is
detected, only zero and double quantum techniques are commonly used. Selection of multiple quantum coherences by phase cycling only, is for in-vivo studies unsatisfactory, because subtraction errors due to motion can be disastrous. Three methods, using gradient pulses to select multiple quantum coherences, are analyzed in this contribution. The double quantum method yields 50% of the maximal signal intensity for AX3 systems. The frequency selective read pulse which is used, has two effects. It doubles the signal intensity (with a non-selective pulse only 25% is obtained) and it suppresses coupled and uncoupled lipid signals. A disadvantage of this method is that two gradient pulses must be optimized, which in-vivo might be difficult. The zero quantum method also yields 50% of the maximal signal intensity, even if the read pulse is not frequency selective. A selective read pulse can be used to suppress coupled and uncoupled lipid signals. Additionally, suppression techniques for signals from uncoupled spins are usually necessary. This can be the suggested T1 cycle, which however has the disadvantage of a two shot experiment. The editing sequence of figure 1e yields 100% of the signal intensity because it smartly refocuses the zero and the double quantum coherences simultaneously. A disadvantage of this sequence is that three gradient pulses must be optimized. Besides this, the sequence heavily depends on a phase cycle to suppress undesired signals from lipids.

With measurements and simulations we showed that the sequences have about the same sensitivity for B1 inhomogeneity. Because this results in a localization effect, the obtained signal to noise ratio is lower than might be expected. The double quantum sequence (1b) and the combined zero plus double quantum sequence (1e) are during the multiple quantum evolution time T1 sensitive to B0 inhomogeneity. It is possible to correct for this by shifting the last 180° pulse in time. Extensions to the multiple quantum techniques using the mqc modulation effects, which yield further editing capabilities, are given.

We think the zero quantum sequence is to be preferred for in-vivo studies of tissues with relatively low lipid concentrations. The
sequence is easy to implement and the zqc frequencies do not depend on the RF frequency. The double quantum method will loose signal intensity if the gradient pulses are not perfectly optimized. If a high lipid suppression is required, or if motion artifacts might occur, the double quantum sequence is favorite. The combined sequence of figure 1e can be used for e.g. post mortem- or extract studies when phase cycling will not give subtraction errors.
For studies on whole body systems on humans, motion errors are smaller because humans mostly do not move as much as animals. For this reason the sequences of figure 1b and 1e might be preferable on whole body systems.

10. REFERENCES


6. DUMOULIN C.L., VATIS D., Suppression of Water and Other Noncoupled


17. BAX A., DE JONG P.G., MEHLKOPF A.F., SMIDT J., Separation of the
Different Orders of NMR Multiple-Quantum Transitions by the Use of
18. KNÖTTEL A., KIMMICH R., Double-Quantum Filtered Volume-Selective
19. NOSEL W., TRIMBLE L.A., SHEN J.F., ALLEN P.S., On the Use of
Double-Quantum Coherence from an AX_3 System (Protons in Lactate)
20. DUMOULIN C.L., The Application of Multiple-Quantum Techniques for
the Suppression of Water Signals in _1^H NMR Spectra, J. Magn.
Reson. 64, 38-46 (1985).
21. DUMOULIN C.L., VATIS D., Water Suppression in _1^H Magnetic Resonance
Images by the Generation of Multiple-Quantum Coherence, Magn.
22. FREEMAN D.M., SOTAK C.H., MÜLLER H.H., YOUNG S.W., HURD R.E., A
Double Quantum Coherence Transfer Proton NMR Spectroscopy
Technique for Monitoring Steady-State Tumor Lactate Acid Levels
23. CROZIER S., BRERETON I.M., ROSE S.E., FIELD J., SHANNON G.F.,
DODDRELL D.M., Application of Volume-Selected, Two-Dimensional
Multiple-Quantum Editing In-Vivo to Observe Cerebral Metabolites,
24. HURD R.E., FREEMAN D.M., Metabolite specific proton magnetic
resonance imaging. Proc. Natl. Acad. Sci. USA 86, 4402-4406
(1989).
Metabolite Specific Method for Determining Tumor Response to Drug
Using Proton Double Quantum Coherence Transfer Spectroscopy, 8th
Annual Meeting of the Society of Magnetic Resonance in Medicine
26. MCKINNON G.C., BOESIGER P., A Robust Method for Localised Lactate
Detection in the Presence of Strong Fat Signals, 8th Annual
Meeting of the Society of Magnetic Resonance in Medicine Abstr.,


34. VAN DIJK J.E., BOVEE W.M.M.J., In-Vivo $^1$H-NMR Spectroscopy of Cerebral Cortex in Rats with Acute Hepatic Encephalopathy or Ammonia Infusion, 9th Annual Meeting of the Society of Magnetic Resonance in Medicine Abstr., p.1042 (1990).


CHAPTER 3.

A Localized In-Vivo Detection Method for Lactate using Zero Quantum Coherence Techniques.

J.E. van Dijk*, D.K. Bosman†, R.A.F.M. Chamuleau*, W.M.M.J. Bovee*

* Delft University of Technology, Delft, The Netherlands.
† Academic Medical Center, Amsterdam, The Netherlands.

Accepted for publication in Magnetic Resonance in Medicine.

Running head: Localized In-vivo Detection of Lactate.

Correspondence to
ABSTRACT

A method is described to selectively measure lactate in-vivo using proton zero quantum coherence techniques. The signal from lipids is eliminated. A surface coil and additionally slice selective localization are used. The resulting spectra demonstrate the good performance of the method.

1 INTRODUCTION.

Line overlap in in-vivo proton spectra makes it difficult to distinguish several metabolites from each other and quantify their concentrations accurately. For this reason it is of great importance to develop techniques which simplify the spectrum or allow selective detection of compounds. With multiple quantum techniques this simplification can be achieved as recently was shown for double quantum techniques (1-5). Several zero quantum sequences have been described. A non-localized zero quantum technique was proposed by Doddrell et al. (6). Their method gives a lactate CH$_3$ intensity of at most 25% of the equilibrium magnetization. Because they used frequency selective gaussian pulses, in combination with a surface coil, they obtained less than 25%. Hall et al. (7,8) described two dimensional zero quantum sequences which are sensitive to motion artifacts ($t_1$ noise) and have an unacceptable long measuring time. Dumoulin (9) investigated the water suppression for zero and double quantum sequences in combination with phase cycling, which is undesirable for in-vivo studies. Recently Trimble et al. (10) demonstrated for lactate, how signals from the double and the zero quantum coherences can be measured simultaneously resulting in a better signal to noise ratio. None of the zero quantum methods mentioned above were used in-vivo. Methods applied successfully in-vitro might be unsuccessful in-vivo because of e.g. motion artifacts or RF inhomogeneity if surface coils are used.
Here we will describe how lactate resonances can be measured selectively in-vivo using a zero quantum technique. Lipid resonances are eliminated, and a good water suppression is obtained. The sequence described here is an adapted version of the STEZQC sequence described by Sotak et al. (11,12), which was also used by Radke et al. (13). A disadvantage of the STEZQC sequence is that the creation of zero quantum coherences depends on the single quantum frequencies. The sequence presented here does not have this disadvantage. Signal contributions from non-coupled spins can be suppressed, not only by subtraction of two spectra as in the STEZQC sequence, but also by a frequency selective read pulse. The sequence can be used with a surface coil in combination with additional localization. Adjustments of inter pulse times and gradient pulses are not necessary. Therefore the sequence can easily be implemented.

2 PRINCIPLES.

The pulse sequence we use is shown in fig 1a and yields 50 % of the maximal $X_3$ intensity for AX$_3$ systems such as lactate and alanine. The 360° pulse and gradient pulses $G_{r2}$ and $G_{r3}$ used for localization will be described later. With the first three RF pulses multiple quantum coherences (mqc's) are created which evolve during $t_1$. After the third RF pulse a gradient pulse is used to dephase all mqc's and transverse magnetizations. Zero quantum coherences (zqc's) are insensitive to this gradient pulse. The read pulse, which can be frequency selective on the CH protons of lactate, converts the zqc's in antiphase transverse magnetizations. The latter evolve into observable transverse magnetizations and reach a maximum at $k/(2J)$ after the fourth RF pulse. $J$ is the scalar coupling constant and $k=1, 3, 5, ...$. The last 180° pulse is used to prevent loss of signal intensities due to static field inhomogeneity effects.

In order to suppress the water signal, a frequency selective adiabatic pulse is used to invert the water resonances. The sequence starts at a
Figure 1. The pulse sequences a) for the zero quantum edited experiment, and b) for the non edited 90-22 experiment.

Using the product operator formalism (15) the following expression is obtained for the signal measured at the $X_3$ frequency during $t_2$ if $\tau_i = k / (4J)$ with $i = 1, 2, 3$ and $4; k = 1, 3, 5, \ldots$:
\[
\frac{1}{2} I_y \cos(2\pi [f_x - f_A] t_1) \cos^2(\pi J t_1) \cos(2\pi f_x t_2) \cos(\pi J t_2) \\
+ \frac{1}{2} I_x \cos(2\pi [f_x - f_A] t_1) \cos^2(\pi J t_1) \sin(2\pi f_x t_2) \cos(\pi J t_2)
\]  

\[1\]

\(f_x\) and \(f_A\) are the resonance frequencies (in Hz) of the X and A spins respectively. \(I_y\) and \(I_x\) are the magnetization components of the X spins measured along the y and x axes.

With the sequence of fig 1a and this choice for \(t_1\), the creation and detection of zqc's is optimized for AX_3 systems. From [1] it follows that the measured signal with frequency \(f_x\) is amplitude modulated with the product of the zero quantum frequency \(f_{zq} = f_x - f_A\) and \(t_1\). The time \(t_1\) can be chosen to optimize the lactate signal intensity. To further eliminate undesired signals from non-coupled protons, two measurements are performed with different \(t_1\) values. The difference in \(t_1\) is \(\Delta t_1 = 1/(2 f_{zq})\). Signals arising from non-coupled spins have a z magnetization during \(t_1\). Therefore they have the same phase in the two measurements. The lactate signal however is 180° out of phase in the second measurement as follows from [1]. After subtraction of the two signals only the lactate signal remains. The \(t_1\) cycle also suppresses the water signal, hence the choice of \(t_o\) is less critical. Because \(\Delta t_1\) is very small (at 7 Tesla \(\Delta t_1 = 0.6\) msec) the effects of the change in \(\cos^2(\pi J t_1)\) and relaxation can be ignored. Of course this is only valid if the ratio unwanted signal to desired signal is not too large. The error resulting from \(J = 7\) Hz is 0.3%, \(T_2 = 100\) msec results in an error of 0.6%. If the lipid suppression of this \(t_1\) cycle is not sufficient, a selective read pulse which only excites the \(x\) proton of lactate can be used. The effect of such a pulse is that the non-coupled spins at 1.3 ppm remain oriented parallel to the z axis, and the zero quantum coherences again are converted in antiphase magnetizations. This selective pulse causes the signal measured during \(t_2\) not to be amplitude, but phase modulated with the product of \(f_{zq}\) and \(t_1\). Of course, this does not influence the effect of the \(t_1\) cycle. Phantom measurements on pure olive oil, using the selective pulse and the \(t_1\)
cycle, showed a CH$_2$ suppression of about 2000 for $\tau_3 = \tau_4 = 34$ msec (second half of echo sampled) and more than 10000 for $\tau_3 = 102$ msec, $\tau_4 = 36$ msec (whole echo acquisition). Using this selective pulse, subtraction errors caused by the $t_1$ cycle are minimized.

The double quantum frequency depends on the carrier frequency and the field strength, the zero quantum frequency only depends on the field strength. So, when zqc sequences are used, the carrier frequency can still be freely chosen and the optimal value for $t_1$ does not have to be redetermined for every experiment. The gradient pulse does not need adjustment because no rephasing is required as is for double quantum coherence techniques. This is of great importance for magnet systems, which suffer from eddy currents. Eddy currents during $t_1$ do not affect the zqc's and hence do not degrade the detected signal. Because of these two arguments the zqc sequence can easily be implemented.

3 RESULTS AND DISCUSSION.

We performed, in-vivo and in-vitro, 2D-ZQC measurements as described by Sotak et al. (11) and found that the complete signal at 1.3 ppm is modulated with the lactate zqc frequency. So no lipid is detected at 1.3 ppm. In fig 1b the pulse sequence of a 90-22 experiment is given which we use to obtain our unedited spectra (16). Typical results of this 90-22 sequence and the zero quantum sequence are shown in fig 2. These measurements are part of our in-vivo study of hepatic encephalopathy (16). All measurements were performed on a home built spectrometer (19) using a surface coil of 11 mm. The lipid peaks in fig 2a & 2c are from two bone ridges on the skull.

At three and four hours after liver ischemia two measurements were performed, first with the 90-22 sequence, immediately followed by the zero quantum measurement. With the zqc sequence the increase of the lactate concentration can be clearly seen (2b,2d), while in the 90-22 spectra (2a,2c) the lactate signal increase is obscured by the lipid
Figure 2. In-vivo brain measurements on a single rat with a surface coil, three (a and b) and four hours (c and d) after liver ischemia. Figures a and c were obtained with the 90°-22 pulse sequence of fig 1, $\tau_1 = 68$ msec, $\delta = 0.6$ msec, $\tau_2 = 18$ msec, $t = 8$ msec, acquisition time = 100 msec, repetition time 4 sec, total duration 160 sec. Spectra d and b were measured with the unlocalized zqc sequence of fig 1a, $\tau_1 = \tau_2 = 34$ msec, $t = 8.75$ msec, $G = 2.0$ Gauss/cm, $t = 4$ msec, $\tau_{1g} = 3/(4J) = 102$ msec, $\tau_4 = 2$ msec (top of echo in the middle of the acquisition time), acquisition time = 200 msec. $G = G = 0$ Gauss/cm. $r_2 = r_3$ The repetition time is 6 sec, the total measuring time is 24 min.
signals. At four hours after liver ischemia the lactate concentration is about 4 mmol (16). It is difficult to compare the signal intensities of the 90-22 spectra and the zero quantum spectra. Not only because the time intervals \( \tau_1 \) in fig 1a and 1b do not correspond, but also because the repetition times differ. Moreover, from simulations and phantom measurements it appeared that the zqc sequence is more sensitive to a deviation of the pulse angles from 90° than the 90-22 sequence. Since surface coils have a spatially dependent \( B_1 \) field, only spins in a region with \( \theta \) close to 90° contribute to the detected signal. The 90-22 sequence is less sensitive to \( B_1 \) inhomogeneity. With this sequence, signals from a larger region are measured and therefore the signal intensity will be higher. Phantom measurements with good RF homogeneity showed that the expected signal intensity of 0.5 \( M_0 \) is obtained with the zqc sequence.

For our in-vivo studies selection of a slice perpendicular to the surface coil is often necessary. For this purpose a localization technique was developed, based on adiabatic slice selective 360° pulses (18), which gives excellent performance with respect to slice profile. A two compartment phantom experiment showed that signal from the outer compartment is more than 2000 times suppressed using this localization technique (19). In fig 3 two brain cortex spectra are shown measured in-vivo on a rat with hepatic encephalopathy. Spectrum 3a was measured without and 3b with the localization pulse selecting a slice of 6 mm width, perpendicular to the rats longitudinal direction. Gradient pulse \( G_{r3} \) is used to compensate for the dephasing of \( G_{r2} \). The lactate concentration changes in time after liver ischemia. Therefore in our experiment the localized and unlocalized sequences were executed alternately. This combined sequence was repeated for signal averaging. In this way both sequences detected the same lactate concentration. The signal reduction is in agreement with the reduced volume which was calculated from spectral images of the water signal.
4 CONCLUSIONS.

A zero quantum coherence pulse sequence is described, which can be used with surface coils. Slice selective pulses provide additional localization. Lactate can be measured in in-vivo situations. Lipid signals are eliminated and a good water suppression is obtained. The sequence can be implemented easily.

![Graph of in-vivo brain spectra of a rat. Spectrum a was measured with sequence 1a as described in the legend to figure 2, spectrum b with: $T_1 = T_2 = 34$ msec, $T_{3a} = 68$ msec, $T_{3b} = 34$ msec, $T_4 = 35.5$ msec, $t_g = 4$ msec, $G_1 = 2.0$ Gauss/cm, acquisition time was 133 msec (whole echo). The duration of the 360 pulse was 9.7 msec, $G_2 = 2$ Gauss/cm. For both spectra the repetition time was 4 sec, the total measuring time per spectrum was 6 min 24 sec.](image-url)
REFERENCES.

(1978).


CHAPTER 4.

Double Quantum- and Spin Echo Sequences for the Selective Detection of Glutamate and Glutamine.

Johannes E. van Dijk, Antoon F. Mehlkopf, Wim M.M.J. Bovee*

Delft University of Technology, Department Applied Physics, section SST/SI, P.O. Box 5046, 2600 GA Delft, The Netherlands.

Submitted to Magnetic Resonance in Medicine and Biology

ABSTRACT

Because the spectral components of glutamate and glutamine severely overlap, their quantification is difficult. Double quantum editing techniques are used at 7 Tesla to selectively detect the γ peak of one of these metabolites and suppress the other. The N-acetyl aspartate peak that overlaps with the γ glutamine peak is 10 times suppressed and the obtained signal intensity is about 15 %. The same glutamine-glutamate selectivity and higher signal intensities are obtained with a two scan spin echo sequence with two echo times. This method suppresses the overlapping N-acetyl aspartate peak only 4 times, and is sensitive to subtraction errors due to motion- and relaxation effects.

KEY WORDS: Double Quantum, Editing, Glutamate, Glutamine

1 INTRODUCTION

With proton Magnetic Resonance Spectroscopy (MRS) it is possible to measure in-vivo signals from several metabolites present in living tissue. One of the difficulties of proton MRS of e.g. the brain is that the obtained spectra consist of many partly overlapping peaks
because of the large number of metabolites that contribute to the detected signal (1,2). A possibility to simplify the spectra is using editing sequences which yield spectra with peaks of only one or a few metabolites. Several editing sequences for AX₃ systems such as lactate have been described. The main goal of these sequences is to suppress the lipid signal and optimally detect the lactate CH₃ signal.

In Figure 1, a typical in-vivo spectrum is shown, measured from the brain cortex of a rat with a surface coil (3). In this Figure can clearly be seen that the lipid and lactate peaks overlap. Two other metabolites that severely overlap are glutamate (GLU) and glutamine (GLN).

Till now there is no procedure presented which makes it possible to measure GLU and GLN selectively. GLU and GLN play an important role in neurotransmission and their quantitative determination would be interesting for studies of neurological disorders (4). Because the overlap of the GLU and GLN peaks makes the quantification of the (relative) concentrations difficult, it is important to measure signals of these neurotransmitters selectively.

There are only a few studies presented in which aspects of the overlapping GLU and GLN peaks are considered. Bates et al (5) used two spin echo sequences with the same echo times, both with a binomial 1331 excitation pulse (6). One sequence has a single 180 pulse, the other sequence has a 2662 binomial 180 pulse. After addition of the measurements obtained with the two sequences, spectra result with the β peaks of GLU and GLN. In this way, the overlapping NAA-CH₃ peak is suppressed and the overlap of GLU and GLN is diminished but still present. McKinnon et al (7) determined the chemical shifts and some coupling constants of GLN. Besides this, they determined with simulations and measurements for GLN how the signal intensity is related to the echo time. They did not try to separate the GLN and GLU signal contributions.

In this work we describe how the γ GLU peak (11 in Figure 1) can be measured without contamination with the γ (GLN) peak (10 in Figure 1) and vice versa. We will refer to this as the selective measurement of
one of these metabolites. To achieve this selectivity we used double quantum coherence sequences and a spin echo sequence with a binomial (22) 180 pulse. The sequences are optimized to give maximal selectivity and signal intensity which for in-vivo application is of great importance. Due to the complexity of the spin systems of GLU and GLN, the optimization process is rather difficult. Because of this we used a semi empirical strategy which will be described further on.

N-acetyl aspartate: 1, 6, 7, 10
Glutamine: 2 (α), 7 (γ), 9 (β)
Glutamate: 2 (α), 8 (γ), 9 (β)
Choline compounds: 3
(Phospho) Creatine: 4
Lipids: 11
Lactate: 12

Figure 1. In-vivo spectrum measured with the sequence of Figure 4c (δ=0.6 msec, T1=68 msec) from the brain cortex of a rat using a surface coil for excitation and detection. The whole echo is sampled.
2 DEFINITION OF THE SPIN SYSTEMS

Analytical calculations which describe the effects of a pulse sequence on the spin system would be of great help by developing the editing sequence. For \(^1\)H MRS the relevant part of the GLU and GLN molecule consists of five protons, see Figure 2. The \(\beta\) protons and the \(\gamma\) protons are all strongly coupled with each other. The \(\alpha\) proton is weakly coupled with both \(\beta\) protons and both \(\gamma\) protons. The coupling between the \(\alpha\) and \(\gamma\) protons is neglected because it is not visible. It

\[
\begin{array}{ccc}
\text{NH}_2 & \text{H} & \text{H} \\
| & | & | \\
\text{C} & \text{C} & \text{C} \\
| & | & | \\
\text{H} & \text{H} & \text{H} \\
\text{(1)} & \text{(2)} & \text{(4)} \\
\alpha & \beta & \gamma
\end{array}
\]

Figure 2. The relevant part of the spin system of glutamate and glutamine. Proton 1 is the \(\alpha\) proton, 2 and 3 are the \(\beta\) protons, 4 and 5 are the \(\gamma\) protons.

It is impossible to perform analytical calculations on a system with 5 unequivalent spins without assuming weak coupling. Results presented below, see Table I, show that such an assumption is not allowed. Therefore we only use numerical calculations which are verified by measurements. Before doing this, all chemical shifts and coupling constants must be known. In the literature we did not find values for these parameters which could be used for our purposes since these are solution dependent (8). Because McKinnon et al (7) determined their values for GLN from measurements on a whole body 1.5 Tesla apparatus, several splittings could not be seen in their spectra and therefore not all coupling constants could be determined accurately. For GLU and
Figure 3. The $\alpha$, $\beta$ and $\gamma$ multiplets obtained from a free induction decay a) glutamine and b) glutamate. At the top the experimental spectra are shown, at the bottom the spectra reconstructed with the parameters given in Table I.
Table I. Chemical shifts and coupling constants of GLU and GLN determined at 7 Tesla. The expected accuracy is 0.1 Hz, for the numbering see Figure 1.

<table>
<thead>
<tr>
<th></th>
<th>GLU (Hz)</th>
<th>GLU (ppm)</th>
<th>GLN (Hz)</th>
<th>GLN (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\omega_1$</td>
<td>305.0</td>
<td>3.78</td>
<td>310.6</td>
<td>3.77</td>
</tr>
<tr>
<td>$\omega_2$</td>
<td>793.2</td>
<td>2.16</td>
<td>799.8</td>
<td>2.13</td>
</tr>
<tr>
<td>$\omega_3$</td>
<td>816.9</td>
<td>2.08</td>
<td>805.0</td>
<td>2.12</td>
</tr>
<tr>
<td>$\omega_4$</td>
<td>724.2</td>
<td>2.39</td>
<td>702.9</td>
<td>2.46</td>
</tr>
<tr>
<td>$\omega_5$</td>
<td>727.2</td>
<td>2.38</td>
<td>711.6</td>
<td>2.43</td>
</tr>
<tr>
<td>J_{12}</td>
<td>4.5</td>
<td></td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>J_{13}</td>
<td>7.2</td>
<td></td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>J_{23}</td>
<td>-15.2</td>
<td></td>
<td>-15.6</td>
<td></td>
</tr>
<tr>
<td>J_{24}</td>
<td>6.0</td>
<td></td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>J_{25}</td>
<td>9.5</td>
<td></td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>J_{34}</td>
<td>8.0</td>
<td></td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>J_{35}</td>
<td>6.8</td>
<td></td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>J_{45}</td>
<td>-15.7</td>
<td></td>
<td>-15.9</td>
<td></td>
</tr>
</tbody>
</table>

GLN we determined the chemical shifts and the coupling constants from a spectrum with the computer program Laocoon4. Spectra ($\Delta\nu_{1/2}=0.9$ Hz) of two model solutions were measured at 7 Tesla, one with 20 mM GLU and one with 20 mM GLN, in water. Both solutions were buffered to pH 7.0, using the buffer Merck 9887. With the determined parameters a free induction decay was simulated. In figure 3 the measured and
simulated α, β and γ multiplets are shown for both spin systems. In Table I the chemical shifts and the coupling constants are given.

Simulations of the editing sequence are performed with a computer program written and given to us by F.J.M. van de Ven, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands. With this program the effects of pulse-, zeeman-, and coupling hamiltonians on spin systems with at most 4 spins, can be simulated. We adapted the program in such a way that calculations on larger spin systems can be performed and we incorporated gradient pulses. Relaxation effects can not be taken into account. The program uses the density matrix formalism to describe the spin system. During a simulation, the effects which the hamiltonians have on the density matrix, are calculated. All simulations are performed with the chemical shifts and coupling constants given in Table I.

3 EXPERIMENTAL

Measurements are performed on a home built spectrometer (9) operating at 7 Tesla using 5 mm NMR tubes. Solutions of GLU and GLN are buffered to pH 7.0 (Merck 9887). In the previous section concentrations of 20 mM were used. In the next sections 20 mM and 30 mM concentrations are used for GLU and GLN respectively. With these concentrations the signal intensity of the γ peaks in the spectra obtained with the spin echo sequence of Figure 4c, is almost equal for GLU and GLN. All measurements are performed with a repetition time of 4 sec. Before conclusions are drawn, results of simulations were always verified with measurements. The simulated and experimental results in general agree. Apart from an illustrative example, only the experimental results are given. For the editing measurements the homogeneity was adjusted to give line widths similar to those obtained in-vivo (~ 0.1 ppm).
Figure 4. Pulse sequences. a) Double quantum sequence without 180 pulses, b) Double quantum sequence with 180 pulses, c) Spin echo sequence with a binomial 180 pulse.
4 RESULTS

4.1 DOUBLE QUANTUM EDITING SEQUENCES

Zero, double, triple or even higher orders of multiple quantum coherence techniques can be used for editing sequences. The selectivity increases, the signal intensity decreases with the order of the mqc's. Since the signal to noise ratio is low in in-vivo MRS, only zero and double quantum coherence techniques are considered. We used double, and not zero quantum sequences because the latter do not sufficiently suppress signals from uncoupled protons (10). As a consequence, zero quantum sequences always have to be combined with water and lipid suppression techniques. Double quantum sequences have proven to adequately suppress signals from non coupled protons, including the water signal. We decided to develop the editing sequences in such a way that the intensities of the γ protons are detected optimally. For this decision several reasons can be given:

1) The γ peaks of GLU and GLN are the only peaks which not completely overlap, so that they can be distinguished in the in-vivo spectra at 7 tesla, see Figure 1.

2) The α peaks are not used because
   a) There are two γ protons and only one α proton. This means that the intensity of the α peaks will be lower.
   b) The α peaks are in the spectrum close to the water peak. Therefore, using the α peaks for editing, high demands on the water suppression are required.
   c) Several amino acids have α peaks which overlap with the α peaks of GLU and GLN.

3) The β peaks are not used because
   a) The two β protons are coupled with each other and with the α and γ protons. This coupling pattern is more complex than that of the γ protons, resulting in lower and more spread out peaks, see figure 3.
   b) The chemical shifts of the two β peaks of GLU differ more than
the chemical shifts of the $\gamma$ peaks. As a consequence, the $\beta$
peaks are more spread out and have a lower intensity than the $\gamma$
peaks, see figure 3.

Besides the selectivity, the most important parameter to be optimized
for the double quantum editing sequence is the signal intensity. In
in-vivo measurements the signal intensities of GLU and GLN obtained
with e.g. a spin echo sequence are low because of the low
concentrations, the effects described before, and J-modulation
effects. This can clearly be seen in Figure 5c and d, the $\beta$ and $\gamma$
peak areas have to be equal because the number of protons (two)
contributing to these peaks is equal. Due to the J-modulation effects
signal contributions cancel. The application of double quantum
techniques will cause further loss of signal intensity because at most
50% of the magnetization contributes to the detected signal but due
to the fact that several different coupling constants are present, the
intensity is lower.

We used the double quantum sequences shown in Figure 4a and b which
are basic double quantum editing sequences often used to edit lactate
(11,12). With the first 90 pulse, transverse magnetizations are
created, which due to the coupling evolve into antiphase
magnetizations (13,14). The latter are partly converted by the second
90 pulse into multiple quantum coherences. The first 180 pulse of
Figure 4b is used to eliminate the effects of the offset frequency and
the zeeman hamiltonians, making the creation of the multiple quantum
coherences of the weakly coupled lactate system independent of the
chemical shifts. After evolving during $t_1$, the mqc's are again partly
converted into antiphase magnetizations by the third 90 pulse.
Hereafter they evolve into observable transverse magnetizations. The
last 180 pulse of Figure 4b creates an echo and reduces signal loss
due to $B_0$ inhomogeneity after the third 90 pulse. The gradient pulses
are used to select the double quantum coherences (15) and suppress
signals from other coherences. Since the double quantum coherences are
twice as sensitive to the gradient pulse as single quantum coherences, they are only rephased if \( G_2 \cdot t_2 = 2 \cdot G_1 \cdot t_1 \). Selection of the double quantum coherences by phase cycle schemes is not considered because in-vivo this technique causes artifacts due to motion, although the obtained signal intensity can be larger.

For \( AX_3 \) spin systems such as lactate, the time \( \tau \) between the first and second 90 pulse usually is taken \( 1/(2J) \), \( J \) being the scalar coupling constant between the A and X spins. The same time lag is used to let the antiphase magnetizations evolve into observable magnetizations before acquisition. Since however the coupling patterns of GLU and GLN are far more complicated and some of the coupling constants are larger than the AX coupling in lactate, the time constant \( \tau \) must be determined otherwise.

The parameters that can be varied to optimize the double quantum sequences of Figure 4a and b are the pulse angles, the pulse phases and the time intervals \( \tau \) and \( t_1 \). The pulse angles are taken 90 and 180 degrees. Since the effects of the 180 pulses on the strongly coupled spin systems is not a priori known, we considered sequences with and without the 180 pulse. If the phase of the second 90 pulse is changed from x to y, other antiphase magnetizations are excited and other double quantum coherences are created. Therefore, sequence 4a and 4b are performed with \( \phi = x \) and \( \phi = y \). Several two dimensional measurements and simulations are performed with different \( \tau \) and \( t_1 \) values. We found that the absolute \( y \) signal intensity is almost constant if \( t_1 \) is only varied in a range of circa 10 msec, starting at 5 msec. The phase of the \( y \) signal component is then modulated with the corresponding double quantum frequency. This can be explained analogous to what is known for \( AX_3 \) systems: the double quantum coherences that contribute to the detected signal evolve during \( t_1 \) due to the zeeman hamiltonian into double quantum coherences which also contribute to the detected signal. This process is the cause of the double quantum phase modulation. Due to the coupling hamiltonians however, the mentioned double quantum coherences evolve also into
Figure 5. a) Double quantum edited glutamine spectrum measured with sequence 4b, \( T = 128 \) msec, \( t_1 = 10 \) msec, 4 scans. b) Double quantum edited spectrum of glutamate measured with the scan parameters of spectrum a). c, d) Spectra of glutamine and glutamate respectively, both measured with sequence 4c, \( \delta = 0.7 \) msec, \( T_1 = 68 \), the whole echo is sampled, 8 scans.
coherences that do not contribute to the detected signal. As long as the change in $t_1$ is much smaller than the time period corresponding to the highest coupling constant, the absolute intensity of the detected signal hardly depends on this change in $t_1$.

Both sequences yield with $\phi=y$ smaller signal intensities as compared to $\phi=x$. Besides this, it appeared that due to the 180 pulses the signal contributions of the double quantum coherences (almost) do not depend on the chemical shifts and the offset frequency.

Simulations and measurements showed that for $\tau$ between 120 and 135 msec. there is an optimum for the signal intensity of GLN and a minimum for GLU. In figure 5a and b spectra are shown measured with sequence 4b, $\tau=128$ msec. For comparison, in figure 5c and d spectra are shown measured from the same solution with sequence 4c. In the edited spectra the GLU $\gamma$ peak intensity is about 50 times suppressed, the intensity of the $\gamma$ peak of GLN is about 20% as compared to the spin echo sequence, see Figure 5c. This means that the ratio of the $\gamma$ GLN and GLU peak areas is 10 times changed in favor of GLN. Unfortunately the same technique can not be used to measure a GLU edited spectrum since there is no value for $\tau$ at which the GLN intensity is (almost) zero.

However, there is an other way to obtain the GLU edited spectrum. This can be done with the sequence of Figure 4b ($\tau=20$ msec) if two measurements are performed with $t_1=11.7$ and $t_1=8.0$ msec. Addition of the two spectra eliminates the GLN $\gamma$ peak but leaves the GLU $\gamma$ peak, see Figure 6. The GLN peak is about 90 times suppressed, the intensity of the GLU peak is 8%, which gives a ratio of only 7.2.

4.2 SPIN ECHO SEQUENCE

Since the double quantum sequences described before gave a dramatic loss of signal intensity and no complete selectivity, we also investigated whether a spin echo sequence can be used to edit GLU and GLN.
The applied sequence is shown in Figure 4c. Because it appeared that the signal amplitude depends in a different way on $\tau$ for GLU and GLN, using sequence 4b, we determined the relation between $\tau_1$ and the $\gamma$ signal intensity of GLU and GLN with sequence 4c. Results are shown in Figure 7. From this Figure it can be concluded that a spectrum with only the GLU peak is obtained if two measurements are performed, with $\tau_1=126$ and $\tau_1=92$ msec and the corresponding spectra are subtracted, see Figures 8a and b. For these values of $\tau_1$ the GLN signal intensity is almost equal, the GLU signal however is maximal and almost zero. A spectrum with only the GLN peak is obtained if the spectra measured

![Figure 6](image_url)

**Figure 6.** a) Double quantum edited glutamate spectrum, using sequence 4b, $\tau=20$ msec, subtraction of signals measured with $t_1=11.7$ msec and $t_1=8.0$ msec, 8 scans. b) Glutamine spectrum measured with the scan parameters of spectrum a).
with $\tau_1 = 60$ and $\tau_1 = 126$ msec are subtracted, see Figures 8c and d. The GLU signal intensities are equal for these values of $\tau_1$ but the GLN intensities are positive and negative. In both cases the loss of signal intensity is about 60%.

Two important disadvantages of this technique are 1) it is a subtraction method which in-vivo can give artifacts due to motion and 2) since the values of $\tau_1$ differ at most 66 msec, effects of relaxation can give subtraction errors. In some cases, the latter effect can be compensated for if relaxation constants are known.

From figure 7 can be seen that maximal signal intensity of GLU and GLN is obtained with the spinecho sequence for $\tau_1 = 65$ msec, even if the sequence is not used for editing.

5 DISCUSSION

The results presented show that it is possible to measure GLU and suppress GLN or measure GLN and suppress GLU, using multiple quantum editing techniques. The obtained signal intensity however is hardly acceptable for application of these techniques in-vivo. With the spin echo sequence higher signal intensities are obtained but in-vivo, motion and relaxation effects might give subtraction errors. For both sequences we investigated whether the $\beta$ peak of N-acetyl aspartate (NAA) that overlaps with the $\gamma$ GLN peak is sufficiently suppressed if a GLN edited spectrum is obtained. The double quantum technique reduces the $\beta$-NAA peak about 10 times as compared to the spin echo sequence with $\delta = 0.6$ msec. The editing method using the spin echo sequence yields a suppression of about 4. So, the double quantum and the spinecho GLN editing sequences suppress the NAA peak 2 and respectively 1.6 times more in favor of GLN. Although this is an improvement, it is no selective detection. The GLU and GLN intensities however can still be determined from the edited spectra. From a non edited spectrum the $\beta$-NAA peak intensity can be obtained using the NAA-CH$_3$ peak intensity which can accurately be estimated by fitting programs. The contribution of the $\beta$-NAA peak to the edited spectrum
can be calculated with the suppression factor of the editing sequence and it can be subtracted from the edited spectrum.

An alternative for the quantification of GLU and GLN is to measure the signal with a non editing sequence and to use a sophisticated data processing technique. This can be a time- or frequency domain fitting procedure that uses spectroscopic prior knowledge to analyze the detected signal and to quantify the (relative) metabolite concentrations. For the detection of lactate, editing techniques are

![Graphs showing the peaks and troughs of various signals as a function of τ₁](image)

**Figure 7.** y peak amplitude (arbitrary units) as a function of τ₁ obtained with sequence 4e, δ=0.7 msec. a) Glutamate measured, b) Glutamine measured, c) Glutamate simulated, d) Glutamine simulated. In the measurements only the second half of the echo is sampled.
the only choice since the lactate peak is completely overlapped by the lipid peaks. At low field strengths editing is also the only choice for GLU and GLN because the overlap is worse than at high field strengths. It must be noticed that at low field strengths the couplings are stronger and modulation characteristics might differ from those presented here. Because the γ GLU and GLN peaks do not completely overlap at high field strengths and the in-vivo application of editing techniques gives several difficulties, a fitting technique is probably preferable.

6 CONCLUSIONS

The chemical shifts and coupling constants of glutamate and glutamine have been determined, see Table I.

Double quantum editing sequences are described which make it possible to separate the overlapping γ peaks of glutamate and glutamine. A disadvantage of these sequences is that the obtained signal intensity is rather low as compared to a spin echo experiment, 20 % for glutamine and 8 % for glutamate. A glutamine edited spectrum can be obtained by using a specific creation and mixing time (128 msec), at which the glutamate signal is about 50 times suppressed. A glutamate spectrum is obtained after subtraction of two signals obtained with different double quantum evolution times (8.0 and 11.7 msec), GLN is than about 90 times suppressed.

It is shown that a spin echo sequence with a binomial 180 pulse can also be used for editing of glutamine and glutamate. Addition or subtraction of signals measured at the appropriated echo times (100, 164, and 232 msec) will give the edited spectra. With this technique the loss of signal intensity is about 60 %. The β N-acetyl aspartate peak that overlaps with the γ glutamine peak is respectively 10 and 4 times suppressed by the double quantum and the spin echo editing techniques. This, and the low signal intensities will make the in-vivo application of these techniques rather difficult.
Figure 8. Edited spectra measured with sequence 4c, only the γ peaks are of importance. a) Glutamate edited spectrum, subtraction of signals obtained with $T_1 = 126$ and $T_1 = 92$ msec, 8 scans. b) Glutamine spectrum measured with the scan parameters of spectrum a). c) Glutamine edited spectrum subtraction of signals obtained with $T_1 = 60$ and $T_1 = 126$ msec, 8 scans. d) Glutamate spectrum measured with the scan parameters of spectrum c).
REFERENCES


10) J.E. van Dijk, W.M.M.J. Bovee, A localized in-vivo detection


CHAPTER 5.

A $^1$H-NMR SPECTROSCOPY STUDY ON THE POSSIBLE PROTECTIVE EFFECT OF L-CARNITINE ON HYPERAMMONEMIA INDUCED ENCEPHALOPATHY IN RATS


*J. van Gool Laboratory for Experimental Internal Medicine, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
^ Department of Applied Physics, Delft University of Technology, Department of Applied Physics SST/SI, P.O. Box 5046, 2600 GA Delft, The Netherlands.

Submitted to Magnetic Resonance in Medicine.

RUNNING HEAD:
$^1$H-NMRS study on the effects of carnitine on encephalopathy

86
ABSTRACT

We studied in rats the possible protective effect of L-carnitine on acute hyperammonemic encephalopathy, induced by administration of ammonium acetate. The only protective effect we observed was a significant shorter increase in EEG left index whereas no improvement was found on the clinical HE grade. NMRS measurements revealed unchanged glutamate concentrations, whereas glutamine increased in both the carnitine and control group. Whether the observed increased (phospho) choline concentration after carnitine administration contributes to the protective effect of carnitine on the EEG is unknown. Increased lactate concentrations, also detected with double quantum editing techniques, were seen in both groups.

1. INTRODUCTION

Hepatic encephalopathy (HE) is a reversible neuropsychiatric syndrome in patients with severe acute or chronic liver function impairment. Ammonia is considered to play a key role in the pathogenesis of this syndrome (1,2). The exact mechanism by which ammonia exerts its neurotoxicity is still matter of debate. Possible neurotoxic effects of hyperammonemia are inhibition of ATP-synthesis, disturbance of neurotransmitter balance and inhibition of neuronal chloride transport (2). Regardless of its neurotoxic mechanism, treatments designed to reverse the hyperammonemia, including neomycin and lactulose, turn out to be effective in the treatment of hepatic encephalopathy (1). A possible new treatment is suggested since O'Connor et al have shown that administration of L-carnitine is able to protect mice against acute ammonia intoxication (3,4). They found that injection of L-carnitine (16 mMol/kg bw i.p.) 30 minutes before a LD100 of ammonium acetate (12 mMol/kg bw) resulted in a 100% survival. Furthermore, injection of L-carnitine 10 minutes after a single dose of ammonium acetate resulted in a 50% reduction of the brain ammonia
concentration. The mechanism by which L-carnitine protects against the neurotoxicity of hyperammonemia is unknown. Several hypotheses have been proposed: restoration of the possibly by hyperammonemia-impaired malate-aspartate shuttle (3), and/or correction of the mitochondrial NADH/NAD+ ratio by increased oxidation of long-chain fatty acids in the presence of increased carnitine levels (5), or neuro-excitatory effects of L-acetyl carnitine on cholinergic cortical neurons based on structural resemblance of L-acetyl carnitine and acetylcholine (6). The promising results of O'Connor et al prompted us to investigate the mechanism of the protective effect of L-carnitine on acute hyperammonemic encephalopathy in the rat by means of in-vivo 1H-NMR spectroscopy. In addition, we quantified the effect of L-carnitine on previously described clinical parameters of HE: EEG spectral analysis, clinical grading and biochemical blood analysis (7).

2. MATERIALS AND METHODS

2.1 ANIMALS

Male Wistar rats (200-275 gr, HSD Zeist, The Netherlands, 12 hr light cycle: 8 a.m.-8 p.m.) were used in all experiments. The animals were fed standard laboratory chow (RM1410, Hope Pharms, The Netherlands) and water ad libitum. Animal care was taken according to the animal welfare guidelines of the University of Amsterdam.

2.2 EEG SPECTRAL ANALYSIS AND CLINICAL GRADING

At least one week before the start of the experiments, four skull electrodes were implanted and EEG analysis was performed. The EEG signal was amplified (Nihon Kohden) and passed through a Butterworth filter (low pass, cut off frequency 50 Hz with a roll off of 48 dB/octave) and analyzed on line. EEG slow wave activity was defined as
the ratio between low frequency power (1.0-7.4 Hz) and high frequency power (13.5-26.4 Hz), the so called EEG left index (8). This EEG left index was used to quantify the EEG abnormalities. The clinical grading of the severity of HE was defined as given in Table 1.

<table>
<thead>
<tr>
<th>grade</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal behaviour</td>
</tr>
<tr>
<td>1</td>
<td>mild lethargy</td>
</tr>
<tr>
<td>2</td>
<td>decreased motor activity, poor posture control</td>
</tr>
<tr>
<td>3</td>
<td>severe ataxia, no spontaneous righting reflex</td>
</tr>
<tr>
<td>4</td>
<td>no righting reflex on painful stimuli</td>
</tr>
<tr>
<td>5</td>
<td>coma, no reaction on painful stimuli</td>
</tr>
</tbody>
</table>

2.3 STATISTICS

Data are given as mean +/- SEM. Significance analysis has been done by Student's t test (9). p-Values below 0.05 are considered to be significant.

2.4 IN VIVO $^1$H-NMRS MEASUREMENTS

Ten rats were studied by $^1$H-NMRS. All NMR experiments were performed at 300 MHz on a home built 7 Tesla spectrometer (10). Three days before the experiments, an ellipsoidal surface coil (axes 14 and 11 mm) was implanted on the skull in order to receive NMRS signals selectively from the forebrain. At regular time intervals localized NMRS signals were obtained with the spin echo sequence of Figure 1a.
Figure 1. NMRS pulse sequences. All time intervals are given in msec.
a) spin echo sequence with two dimensional localization, $\tau_1 = 68$, $\delta = 0.6$, $\tau_2 = 18$, $G_z = 20$ mT/m. 
b) Double quantum sequence selecting the double quantum coherences with gradient pulses. $\tau_0 = 68$, $t_1 = 10$, $\tau_1 = 102$, $\tau_2 = 31.25$, $t_1 = 6$, $t_2 = 12$, $G_1 = G_2 = 10$ mT/m (z direction). Spool gradients, not shown, (x direction 10 mT/m) around the first 180 pulse. Exor phase cycling is performed on the binomial pulse of sequence a and on the last 180 pulse of sequence b.
The animals are not anesthetized during the NMR experiments. They are held in position by a head-body holder (11). Shimming was performed over the entire volume.

The water signal is suppressed with the binomial 180 pulse (12). The interpulse time δ is chosen to optimally detect the NAA-CH₃ signal at 2.01 ppm. Localization was performed as described elsewhere (13) selecting a region of 6 x 9 x 3 mm. Due to this localization, lipids are suppressed to such an extend that the lactate peak at 1.3 ppm which usually is severely overlapped by lipid peaks, is clearly visible. However, it is still contaminated with lipids. Therefore the lactate signal is also measured with a double quantum editing sequence (Figure 1b), immediately after the spin echo sequence of Figure 1a.

The double quantum editing sequence is developed to selectively measure lactate and suppress lipid (14). During τ₀ the double quantum coherences are created and they evolve during τ₁. With the last 90 pulse, which only excites the CH proton of lactate, they are converted into transverse magnetizations. Due to the last 180 pulse an echo-like signal is detected. The double quantum coherences are selected with the gradient pulses (15). Although the double quantum sequence suppresses the water signal, this signal is further reduced with a frequency selective pulse which inverts the water resonances. The sequence starts at a time t₀ after this pulse; t₀ is chosen to minimize the remaining water signal (16).

Quantification of the spin echo measurements was performed with a fitting procedure as described previously (17). The double quantum spectra were quantified by integration.

2.5 EXPERIMENTAL PROTOCOL

Series A was performed in order to quantify the effect of L-carnitine administration on EEG spectra, clinical grading and plasma ammonia, whereas series B was used for the ¹H-NMR spectroscopy study.
SERIES A:
Sixteen hours before T=0 all rats were injected i.p. with urease (1000 U/kg bw). Two hours before the start of the experiment two cannulas were inserted into respectively the carotic artery and the peritoneal cavity. Thirty minutes before the start of the experiment the rats were injected with L-carnitine (8 or 16 mMol/kg bw i.p.) or an equivalent amount of saline. On T=0 the rats were injected with ammonium acetate (7 mMol/kg bw). Blood (0.5 ml) was sampled from the carotic cannula for measuring the plasma ammonia concentration at different time intervals as indicated.

SERIES B:
In principle the same protocol was used as for series A, only no cannulation of the carotic artery was performed. Furthermore, only the effect of 16 mMol/kg bw i.p. carnitine was studied in 5 rats, in comparison to 5 control rats.

Figure 2: Acute hyperammonemia. Carnitine effects on plasma ammonia.
(* means p<0.05)
3. RESULTS

SERIES A

The plasma ammonia concentrations of the saline and carnitine treated rats are shown in Figure 2. Only in the presence of 16 mMol/kg L-carnitine a significant decrease in plasma ammonia is observed at 10 and 20 min.

The EEG left index both in the absence and presence of L-carnitine is shown in Figure 3. Administration of L-carnitine in the absence of hyperammonemia did not influence the EEG left index (not shown). A dose of 16 mMol/kg bw L-carnitine results in a normalization of the left index in the period 30-120 min after ammonium acetate administration, whereas 8 mMol/kg L-carnitine showed values in between saline and L-carnitine (16 mMol/kg) treatment.

Unfortunately this EEG improvement is not reflected in the clinical grading (Figure 4). The development of the clinical grade of HE was even more rapid in the L-carnitine 16 mMol/kg group in the first 45 min. Thereafter clinical HE grade was similar in all groups. Within 5

![Graph showing EEG left index over time for saline and carnitine groups.](image)

**Figure 3:** Acute hyperammonemia. Carnitine effects on EEG left index. The EEG left index is presented as percentage of normal values.
Figure 4: Acute hyperammonemia. Carnitine effects on clinical grading.

to 20 minutes the rats became drowsy and reached grade 3 of HE for another 90 min.

SERIES B
Quantification of NMR spectra of normal rats receiving carnitine, revealed no differences in all NMRS parameters studied. This indicates that the administration of carnitine alone is ineffective in inducing changes in the substances measured by NMRS.
The effect on NMRS glutamate is shown in Figure 5. Both control and carnitine treated rats did not show any significant changes in the cerebral cortex glutamate concentration. Control rats (Figure 6) showed significant increased glutamine concentrations from 15 to 90 min after the ammonium acetate administration. In the carnitine treated rats glutamine was only significantly increased after 30 min. Compared to normal values, both groups showed an significant increase in lactate at T = 15 min. At T =30 min only the carnitine group showed significantly increased lactate concentrations. After 45 min in both
groups studied, lactate had rapidly declined to near normal values (Figure 7). The double quantum lactate measurements showed analogous results (Figure 8). In the control group a significant decrease of the (phospho) choline compounds (Figure 9), as detected by NMRS, is seen at \( T = 60 \) and 90 min. In the carnitine treated group, however, a significant increase (compared to both normal values and control group values) was observed already after 15 min, lasting throughout the whole experiment.

**NMRS GLUTAMATE**

- Controls
- Carnitine

**Figure 5:** The percentual changes of Glutamate for the control- and carnitine treated rats.
4. DISCUSSION

The striking protective effect of L-carnitine against the toxicity of hyperammonemia in mice as shown by O'Connor et al (3,4) has not been confirmed in our experiments in rats. Only a protective effect on the EEG slow wave activity could be observed in acute hyperammonemia: the

**NMRS GLUTAMINE**

![Graph showing glutamine concentration over time](image)

Figure 6: The percentual changes of Glutamine for the control- and carnitine treated rats.

increase in EEG left index after ammonium acetate administration was significantly shorter of duration in the treated group. In contrast to saline treated animals the EEG left index normalized within 30-45 min, whereas peak levels of plasma ammonia were significantly less during the first twenty minutes in the presence of L-carnitine (16 mMol/kg). A lower dose of L-carnitine (8 mMol/kg) induced EEG left index changes in between saline-treated and high-dose carnitine rats. Unfortunately this improvement of the EEG by L-carnitine during acute hyperammonemia was not reflected in the clinical HE-grade: up to 2 hrs after acute
ammonium acetate loading, the animals were still in coma grade 2. These observations are in agreement with those of Hearn et al (18) who also showed a variable protective effect of L-carnitine (16 mMol/kg bw) administered 1 hr prior to a LD100 of ammonium acetate in rats. This variable protective effect of L-carnitine was also short-lived and completely lost if ammonium acetate was given 24 hrs after

**NMRS LACTATE**

![Graph showing lactate concentration over time](image)

**Figure 7:** The percentual changes of Lactate for the control- and carnitine treated rats measured with the spin echo sequence of Fig 1a.

L-carnitine administration.

The strong increase in cerebral cortex lactate, observed in both groups with all three sequences, can be explained by simultaneous ammonia induced stimulation of phosphofructokinase activity and inhibition of pyruvate dehydrogenase and has been described before (2,19,20). Since the ammonia induced increase in cerebral cortex lactate was similar in both groups of rats, a protective effect of carnitine on the cytosolic redox state, as suggested by O'Connor (3,4), is
unlikely. The unchanged NMRS glutamate and the increased glutamine concentrations observed, can be explained by inhibition of glutaminase activity rather than an increased glutamine synthesis as suggested by Cooper et al (2). The observation that in carnitine treated rats a significant increase of glutamine was found at T=30 min, whereas control rats showed this increase at T=15 min, might be related to the reduced plasma ammonia levels seen at the same time intervals after carnitine administration.

Another explanation for the protective effect of carnitine could be the significant increase in (phospho) choline compounds. Since we previously described that both acute hepatic encephalopathy and sub-acute hyperammonemia are associated with a significantly decreased (phospho) choline concentration (18), the induced increase could exert some protective effect as is possibly reflected in the parallel observed improvements on EEG spectral analysis. It should be stated, however, that since the exact origin of these compounds is still unknown, such an explanation is speculative.

**NMRS DOUBLE QUANTUM**

--- Controls  --- Carnitine

![Graph showing peak area over time](image)

**Figure 8**: The changes of Lactate for the control- and carnitine treated rats measured with the double quantum sequence of Figure 1b.
Figure 9: The percentual changes of (Phospho) Choline for the control- and carnitine treated rats.

Furthermore, alternative explanations such as interferences of L-carnitine with the ammonia-induced decreased inhibitory post synaptic potential, can not be excluded. Although L-carnitine has a small peripheral effect in acute hyperammonemia in rat (series A) as shown by a small but significant decrease in plasma ammonia concentration in the first 20 min, it is very unlikely that this observation could explain the central effect: EEG left index normalized in the L-carnitine 16 mMol/kg group whereas plasma ammonia (and therefore brain ammonia) was still in a neurotoxic range (more than 1000 uM) (2).

Finally, it remains unexplained why the protective effect of L-carnitine is much more pronounced in mice than in rats. More studies are needed to answer all these questions.
5. REFERENCES


CHAPTER 6.

DETERMINATION OF CONCENTRATIONS BY TIME DOMAIN FITTING OF PROTON NMR ECHO SIGNALS USING PRIOR KNOWLEDGE


RUNNING HEAD:
Time domain fitting of nmr signals using prior knowledge.

* To whom correspondence should be addressed.

Department of Applied Physics., section SST/SI, Delft University of Technology, P.O. Box 5046, 2600 GA Delft, The Netherlands.

104
ABSTRACT

A fast and flexible time domain iterative fitting procedure is described that can be used to fit free induction decays as well as echo-like signals. Damping constants of the first and second part of the echo do not have to be identical. Prior knowledge can be used to diminish the number of parameters to be fitted, which results in an improved accuracy. It is shown how prior knowledge is mathematically incorporated in the Gauss-Newton method. From proton NMR measurements of model solutions actual prior knowledge is extracted. With this knowledge relative concentrations are determined from a mixture of metabolites. The fitted results agree with the true values within the margins of the noise. After some minor changes the same prior knowledge was successfully used to analyze a series of in-vivo rat brain measurements.

1. INTRODUCTION

For in-vivo proton MRS studies it is often important to quantify (relative) concentrations of metabolites and small changes of these concentrations as a function of time. Determination of peak areas by integration is an established technique (1,2). If lines overlap, accurate quantification of proton spectra becomes difficult. A good possibility to improve the accuracy of the quantification is the use
of smart algorithms that combine smoothing and estimation of signal parameters as e.g. (3). Another possibility is fitting model functions to the spectrum or to the time domain signal. Fitting the spectrum has proven to give good results (4,5), but the Fourier transform may introduce distortions in the spectrum (6) and the subsequent quantification due to truncation.

Most time domain quantification methods, see e.g. (7-16), are based on free induction decays (FID's). However, using proton MRS techniques, often echo-like signals are detected. After truncation of the first half of the echo, the resulting FID-like signal can be used to determine signal parameters such as amplitudes, damping constants, frequencies, and phases. This however implies that a significant number of data points with a relatively high signal to noise ratio are omitted. Especially for in-vivo proton measurements with low S/N ratios, a quantification method that uses all data points is favorable. Here we describe the application of a variant of non-linear least squares model fitting, namely the Gauss-Newton method, see e.g. (16). Provided one entertains the exponentially damped sinusoids model, this method is particularly able to reduce the computational burden attendant to non-linear least squares methods (9). In addition, it is easy to implement prior knowledge into the Gauss-Newton method. The developed computer program is an intelligent procedure that, for a given problem and set of known parameters/relations, constructs the appropriate reduced system of equations.

In this work we emphasize how prior knowledge is obtained and imposed.
Although in this contribution the aspects of imposing prior knowledge are considered in the time domain, the method is applicable in any domain. Note that the Bayesian estimation method which recently is described (17), is also suitable for incorporation of prior knowledge, in any domain.

The basic principles of the Gauss-Newton method are described in section 2. Relations between parameters, a priori known, can be used to diminish the number of parameters to be fitted. This not only reduces the computing time, but also the so-called Cramér Rao lower bounds on the standard deviations of quantified parameters (18). The incorporation of prior knowledge is discussed in section 3. Experimental verification is described in section 4. First it is shown how prior knowledge can be obtained from measured data. Hereafter, this knowledge is used to fit in-vitro and in-vivo measured signals consisting of several sinusoids that severely overlap in the frequency domain.
2. INTRODUCTION OF THE THEORY

The theory of the fitting procedure for FID signals is described elsewhere (10). Here the signal is expected to be an echo, consisting of \( K \) exponentially damped sinusoids. The echo is modeled as two FID's, back to back. The damping constants of the forward and backward components of the sinusoids do not have to be equal. This makes it possible to incorporate effects of relaxation without special

Figure 1. The effect of transverse relaxation on the measured echo.

--- The envelope of the echo without \( T_2 \) relaxation effects.

--- Signal decrease due to relaxation.

--- The envelope of the echo with \( T_2 \) relaxation effects.
precautions. The echo is symmetric if the transverse decay rate $T_2^{-1}$ can be neglected with respect to the rate due to the magnetic field inhomogeneity $T_{2m}^{-1}$. Otherwise the effects of $T_2$ on the intensity of the transverse magnetization $M_{tr}$, shown in Figure 1 and Eq. [1], must be taken into account:

$$M_{tr}(t) = M_0 \exp(-t/T_2) \exp(-(t_0-t)/T_{2m}) \quad t_b < t < t_0$$

$$= M_0 \exp(-t/T_2) \exp((t_0-t)/T_{2m}) \quad t \geq t_0$$  \hspace{1cm} [1]$$

$M_0$ is the transverse magnetization at $t=t_b$.

The time origin is chosen at the transition from the backward FID to the forward FID, see Figure 2. Note that the top of the experimental echo does not have to be in the middle of the sampling period and it does not have to coincide with a sample point. The latter is taken into account by introducing the quantity $\Delta$, $0 \leq \Delta \leq 1$. The model function $\hat{x}$, used by the algorithm is given by Eq. [2].

![Diagram](image)

*Figure 2. Definition of the time axis used in the fitting algorithm.*
\[
\xi_n = \left\{ \begin{array}{ll}
\sum_{k=1}^{K} c_k \exp \left( (\xi_k + i\omega_k) t_n + i(\phi_0 + \phi_k) \right) & n = -N, \ldots, 0 \\
\sum_{k=1}^{K} c_k \exp \left( (\xi_k + i\omega_k) t_n + i(\phi_0 + \phi_k) \right) & n = 1, \ldots, M
\end{array} \right.
\]

In Eq. [2] \( \xi_k \) stands for the backward damping constant of sinusoid \( k \), \( \zeta_k \) for the forward damping constant, \( \omega_k \) for the frequency, \( \phi_k \) for the phase, and \( c_k \) for the amplitude. The overall phase is denoted by \( \phi_0 \); \( t_n = -N + \Delta, \ldots, M + \Delta \). The time between two successive data points is normalized to 1. The phase factors \( \phi_k \) and \( \phi_0 \) as well as \( \Delta \) are not essential for the incorporation of the sort of prior knowledge as considered here, and are ignored. Consequently, \( t_n \) is put equal to \( n \). The simplified model function used in this work is given in Eq. [3].

\[
\xi_n = \left\{ \begin{array}{ll}
\sum_{k=1}^{K} c_k \exp \left( (\xi_k + i\omega_k) n \right) & n = -N, \ldots, 0 \\
\sum_{k=1}^{K} c_k \exp \left( (\xi_k + i\omega_k) n \right) & n = 1, \ldots, M
\end{array} \right.
\]

The Gauss-Newton approach amounts to linearizing the dependence of the model function on the parameters, by putting

\[
\begin{align*}
\xi_k &= \xi_k' + \delta\xi_k \\
\zeta_k &= \zeta_k' + \delta\zeta_k \\
\omega_k &= \omega_k' + \delta\omega_k
\end{align*}
\]

in which \( \delta\xi_k \), \( \delta\zeta_k \), and \( \delta\omega_k \) are assumed small.
The parameters $\xi'_k$, $\zeta'_k$, and $\omega'_k$ are starting values or results of the previous iteration. The algorithm calculates changes of $\xi'_k$, $\zeta'_k$, and $\omega'_k$, which are denoted by $\delta\xi'_k$, $\delta\zeta'_k$, and $\delta\omega'_k$. In Eq.[5] the linearized version of Eq. [3] is given, which in principle is the first order Taylor approximation.

\[
\begin{align*}
\bar{x}_n &= \left\{ \begin{array}{ll}
\Sigma & (c_k + n\delta\xi'_k c_k + i n \delta\omega'_k c_k) \exp((\xi'_k + i\omega'_k)n) \quad n = -N, \ldots, 0 \\
-k & \Sigma (c_k + n\delta\zeta'_k c_k + i n \delta\omega'_k c_k) \exp((\zeta'_k + i\omega'_k)n) \quad n = 1, \ldots, M
\end{array} \right.
\end{align*}
\]

[5]

Defining the $(N+1)\times K$ matrix $\bar{F}_\xi$ and the $M\times K$ matrix $\bar{F}_\zeta$ as follows

\[
(\bar{F}_\xi)_{n,k} = \exp((\xi'_k + i\omega'_k)n), \quad n = -N, \ldots, 0 ; \quad k = 1, \ldots, K
\]

[6]

\[
(\bar{F}_\zeta)_{n,k} = \exp((\zeta'_k + i\omega'_k)n), \quad n = 1, \ldots, M ; \quad k = 1, \ldots, K
\]

Eq. [5] can be written in matrix form using the Hadamar product $\otimes$ (19), which is more convenient and illustrative:

\[
\begin{pmatrix}
\bar{x} \\
\bar{\zeta}
\end{pmatrix}
= \begin{pmatrix}
\bar{F}_\xi & n\bar{F}_\xi & 0 & i n \bar{F}_\xi \\
\bar{F}_\zeta & 0 & n\bar{F}_\zeta & i n \bar{F}_\zeta
\end{pmatrix} \otimes \begin{pmatrix}
c \\
\delta\xi' \\
\delta\zeta' \\
\delta\omega'
\end{pmatrix}
\]

[7]

\[
\bar{x} = \begin{pmatrix}
\bar{x}_{-N} \\
\vdots \\
\bar{x}_0
\end{pmatrix}, \quad \bar{\zeta} = \begin{pmatrix}
\bar{x}_1 \\
\vdots \\
\bar{x}_M
\end{pmatrix}, \quad \bar{c} = \begin{pmatrix}
c_1 \\
\vdots \\
c_K
\end{pmatrix},
\]

and $\delta\gamma\bar{c} = \begin{pmatrix}
\delta\gamma_1 c_1 \\
\vdots \\
\delta\gamma_K c_K
\end{pmatrix}$, $\gamma$ being $\xi$, $\zeta$, or $\omega$. 

111
From Eq. [7] it can clearly be seen which parts of the matrices and vectors pertain to the amplitudes, damping constants and frequencies respectively.

The least squares solution of Eq. [7] can easily be derived (18). To this end, Eq. [7] is rewritten in condensed form as:

$$\bar{x} = \bar{F} \bar{p}$$  \hspace{1cm} [8]

with the data vector $\bar{x}$, and $\bar{p} = \begin{pmatrix} \delta \xi & \xi \\ \delta \zeta & \zeta \\ \delta \omega & \omega \end{pmatrix}$ being the parameter vector to be estimated. The matrix $\bar{F}$ is defined as follows:

$$\bar{F} = \begin{pmatrix} \bar{F}_{\xi} & \bar{n}_{\xi} & \bar{i}_{\xi} \\ \bar{F}_{\zeta} & \bar{n}_{\zeta} & \bar{i}_{\zeta} \end{pmatrix}.$$  

The standard least squares solution of Eq. [7] is (18):

$$\bar{p} = (\bar{F}^+ \bar{F})^{-1} \bar{F}^+ \bar{x} = \bar{U}^{-1} \bar{A}$$  \hspace{1cm} [9]

with $\bar{U} = \bar{F}^+ \bar{F}$ and $\bar{A} = \bar{F}^+ \bar{x}$.

The symbol $^+$ denotes Hermitian conjugation.

From Eq. [9] new values for $c_k$, $\delta \xi_k$, $\delta \zeta_k$, and $\delta \omega_k$ are obtained. With these values $\xi_k$, $\zeta_k$, and $\omega_k$ are updated and a new iteration step is started. The iteration process is controlled by the following, empirically obtained restrictions: the amplitudes and damping
constants are at most changed with a factor 2, the frequency of a sinusoid is not changed beyond the line width. The procedure is fast in the sense that a special property of exponentially damped sinusoids is exploited. The property alluded to is \( \mathbf{F}_{n+1,k}^\alpha = z^\alpha \mathbf{F}_{n,k}^\alpha \) for all \( n \). In this relation \( z = \exp(\alpha + i\omega) \) and \( \alpha = \xi \) or \( \alpha = \zeta \). Using this relation, an analytical formula can be derived for each element of the matrix \( \mathbf{F}^\top \mathbf{F} \) in Eq. [9]. The same property is used for the calculation of \( \mathbf{F}^\top \mathbf{x} \). For more details see reference (10). We found that the described procedure is about 30 times faster as compared to the VARPRO program (20). It must be noticed that these measures can also be helpful in other existing non-linear least squares procedures. The overall phase factors \( \phi_0 \) and \( \Delta \) are fitted in the same manner as the other non-linear parameters \( \xi_k, \zeta_k, \) and \( \omega_k \). The phases \( \phi_k \) usually do not have to be fitted. However, if necessary, fitting of the individual phases can easily be incorporated.

3.1 INCORPORATION OF PRIOR KNOWLEDGE

In general one should estimate as few parameters as possible to improve the goodness of the estimates and the convergence rate. Thus, if a relation between two or more parameters is known, this knowledge should be used to diminish the number of parameters which have to be fitted. In the theory described before, this results in a reduction of the dimensions of the matrix \( \mathbf{F} \) and the vector \( \mathbf{p} \). In the actual
computer program the reduction formulae are applied on $\bar{p}$, $\bar{U}$ and $\bar{A}$ of Eq. [9]. For readability, in the next section is shown, how a reduced matrix $\bar{F}$ and vectors $\bar{x}$ and $\bar{p}$ can be obtained if a relation between amplitudes is known, while keeping the other parameters fixed. For prior knowledge concerning frequencies and damping constants analogous relations are described in appendix 1 and 2 respectively. Note that all free parameters can be fitted concurrently. This merely involves larger matrices and vectors.

3.2 PRIOR KNOWLEDGE ABOUT AMPLITUDES

In this subsection only the amplitude part of Eq. [7] is considered. This is permitted if $\delta\bar{c} = \delta\bar{c} = \delta\bar{c} = \bar{0}$. Distinction between the $\xi$ and $\zeta$ part is not essential here and is therefore omitted. Two kinds of prior knowledge are distinguished:

1) For two different peaks $k$ and $l$ of the same spin system, it is often possible to derive a relation between the two amplitudes:

$$c_l = a \cdot c_k$$

To show how this knowledge leads to a reduced matrix $\bar{F}$ and vector $\bar{p}$, the amplitude part of Eq. [5] is rewritten:
\[
\begin{pmatrix}
x_{-N} \\
\vdots \\
x_M
\end{pmatrix} = 
\begin{pmatrix}
F_{-N,1} & \cdots & F_{-N,k} & \cdots & F_{-N,\ell} & \cdots & F_{-N,K} \\
\vdots & & \vdots & & \vdots & & \vdots \\
F_{M,1} & F_{M,k} & \cdots & F_{M,\ell} & \cdots & F_{M,K}
\end{pmatrix}
\begin{pmatrix}
c_1 \\
c_K \\
\vdots \\
c_{\ell} \\
ac_K \\
c_K
\end{pmatrix}
\]

\[
= 
\begin{pmatrix}
F_{-N,1} & \cdots & F_{-N,k} & \cdots & F_{-N,\ell} & \cdots & F_{-N,K} \\
\vdots & & \vdots & & \vdots & & \vdots \\
F_{M,1} & F_{M,k} & \cdots & F_{M,\ell} & \cdots & F_{M,K}
\end{pmatrix}
\begin{pmatrix}
c_1 \\
c_K \\
\vdots \\
c_{\ell} \\
ac_K \\
c_K
\end{pmatrix}
\]

\[
= 
\begin{pmatrix}
F_{-N,1} & \cdots & F_{-N,k} + a F_{-N,\ell} & \cdots & F_{-N,K} \\
\vdots & & \vdots & & \vdots \\
F_{M,1} & \cdots & F_{M,k} + a F_{M,\ell} & \cdots & F_{M,K}
\end{pmatrix}
\begin{pmatrix}
c_1 \\
c_K \\
\vdots \\
c_{\ell} \\
c_K
\end{pmatrix}
\rightarrow
\]

\[
\hat{x} = \frac{F \cdot c}{c_K}
\]

Here the subscript \( R \) is used to indicate that the variable is reduced. Further on it will also be used on \( \hat{x} \) to indicate that a column of \( \overline{F} \) is subtracted from \( \overline{\tilde{x}} \). Columns \( \ell \) and \( k \) of \( \overline{F} \) are concatenated, and element \( \ell \) of \( \overline{c} \) is eliminated. Amplitude \( c_{\ell} \) is not calculated during the iterative process, but afterwards, using the equation \( c_{\ell} = a c_K \).
Notice that due to the implementation of prior knowledge in the fitting procedure, the proportional error bounds of related amplitudes are equal.

2) In some cases an amplitude is known: \( c_k = \alpha \). Now it follows that:

\[
\begin{bmatrix}
\omega \\
\vdots \\
\omega
\end{bmatrix} =
\begin{bmatrix}
F_{-N,1} & \ldots & F_{-N,k} & \ldots & F_{-N,K} \\
\vdots & & \vdots & & \vdots \\
F_{M,1} & \ldots & F_{M,k} & \ldots & F_{M,K}
\end{bmatrix}
\begin{bmatrix}
c_1 \\
\vdots \\
c_k \\
\vdots \\
c_K
\end{bmatrix}
\]

\[
= \begin{bmatrix}
F_{-N,1} & \ldots & F_{-N,k} & \ldots & F_{-N,K} \\
\vdots & & \vdots & & \vdots \\
F_{M,1} & \ldots & F_{M,k} & \ldots & F_{M,K}
\end{bmatrix}
\begin{bmatrix}
c_1 \\
\vdots \\
\alpha \\
\vdots \\
c_K
\end{bmatrix}
\]

\[
= \begin{bmatrix}
F_{-N,1} & \ldots & F_{-N,K} \\
\vdots & & \vdots \\
F_{M,1} & \ldots & F_{M,K}
\end{bmatrix}
\begin{bmatrix}
c_1 \\
\vdots \\
c_K
\end{bmatrix}
+ \alpha \begin{bmatrix}
F_{-N,k} \\
\vdots \\
F_{M,k}
\end{bmatrix}
\]

\[
\Rightarrow \begin{bmatrix}
\omega \\
\vdots \\
\omega
\end{bmatrix} = \begin{bmatrix}
F_{-N,k} \\
\vdots \\
F_{M,k}
\end{bmatrix} = \begin{bmatrix}
F_{-N,1} & \ldots & F_{-N,K} \\
\vdots & & \vdots \\
F_{M,1} & \ldots & F_{M,K}
\end{bmatrix}
\begin{bmatrix}
c_1 \\
\vdots \\
c_K
\end{bmatrix}
\]

\[
\Rightarrow \begin{bmatrix}
\omega \\
\vdots \\
\omega
\end{bmatrix} = \begin{bmatrix}
F_{R} \\
\vdots \\
F_{R}
\end{bmatrix}
\begin{bmatrix}
c_R \\
\vdots \\
c_R
\end{bmatrix}
\]

The contribution of sinusoid \( k \) (using \( \xi_k' \), \( \zeta_k' \), and \( \omega_k' \)) is subtracted.
from the data vector. In summary, the recipe is that amplitude \( c_k \) is not calculated and therefore column \( k \) can be eliminated from \( \tilde{F} \). The known amplitude multiplied by the eliminated column of \( \tilde{F} \) is subtracted from the data vector \( \tilde{x} \).

4. EXPERIMENTAL VERIFICATION

In our in-vivo proton MRS study of hepatic encephalopathy, we are interested in changes of rat brain cortex concentrations of metabolites such as glutamine (GLN), glutamate (GLU), aspartate (ASP), and N-acetyl aspartate (NAA). In the in-vivo spectra, peaks of these metabolites severely overlap. Because of this overlap, reproducible and reliable quantification of metabolite concentrations is only possible if a model function is fitted to the signal.

In the first part of this section (4.1 and 4.2) the time domain program, described in section 2., is used to quantify signals from model solutions of the previously mentioned metabolites. The theory described in 3. is used to diminish the number of parameters to be fitted.

In the second part of this section (4.3 and 4.4) the same method is used to analyze a series of in-vivo measurements. Finally some remarks are given in 4.5.

All measurements are performed with a home-built spectrometer operating at 7 T, using an ellipsoidal surface coil with axes of 14
and 11 mm with the sequence of Figure 3. The $\theta_x - \theta_{-x}$ pulse is a 180° binomial refocusing pulse (21) which suppresses also the water signal. The interpulse time $\delta$ equals 0.6 msec and is chosen such that the CH$_3$ resonance of NAA at 2.01 ppm rotates exactly 180° during $\delta$. Therefore this resonance is perfectly refocused by the binomial pulse and optimally detected. The FID, created by the last RF pulse is destroyed by the gradient pulse and further eliminated by the EXOR phase cycling scheme (22). In-vivo, additional localization besides the surface coil is necessary to eliminate intense lipid signals and to improve the water suppression.

Both the long axis of the surface coil and the body axis of the rat are along the z-direction. The short axis of the coil is along the x-direction (ear to ear). The depth into the brain corresponds to the y-direction. The first $\theta$ pulse of Figure 3 is a sinc-Gauss amplitude

![Diagram](image)

Figure 3. Spin echo sequence for in-vivo use which suppresses the water signal. G: homogeneity spoil gradient pulse, 20 mT/m. The flip angle $\theta$ is 150° in the center of the surface coil; $\tau_1 = 68$ ms; $t_1 = 4$ ms; $\delta = 0.6$ ms; $\tau_2 = 18$ ms; acquisition time $t_2 = 100$ ms; and repetition time 4 s, 768 quadrature data points are sampled. Exor phase cycling of the binomial pulse is used to correct for pulse imperfections.
modulated pulse of 2.5 ms duration (three lobes in total), which is applied in the presence of a 20 mT/m magnetic field gradient (not shown) and selects an x-y-slice of 6 mm thickness perpendicular to the surface coil. Measurements on the model solutions are performed with a rat brain phantom under in-vivo conditions. The procedure followed in this section is analogous to the one of De Graaf et. al. (4) who fitted in the frequency domain.

4.1 OBTAINING PRIOR KNOWLEDGE IN-VITRO

Model solutions in water of ASP, GLU, GLN, and NAA were prepared, see Table 1. All solutions were buffered to pH 7. After the measurements, all signals were corrected by the QUALITY method (23). Regardless of the static magnetic field inhomogeneity, pure Lorentzian line shapes are obtained by division of the experimental NMR time domain signal by a reference signal. Hereafter, Lorentzian time domain windowing was applied with a time constant of 50 msec to improve the S/N ratio and for reasons mentioned below. Finally the signals of the four solutions were fitted. By giving good starting values for the frequencies which are obtained with a peakpick method, the program gave only solutions in the interesting frequency region between 1.5 and 3 ppm. In the in-vivo-like spectra at 7 T, multiplet structures, that can be seen in high resolution spectra, are not always visible because of line broadening. Even after applying QUALITY on in-vivo signals with a
Figure 4. The spectra of the experimental (solid lines) and fitted (dotted lines) signals of the separate solutions, a) 40 mM NAA, b) 20 mM ASP, c) 40 mM GLN, and d) 40 mM GLU. Gaussian time domain windowing was applied with 21 ms before the FFT as is done with in-vivo measurements.
Figure 4. (Continued).
separately measured reference signal, the multiplet structures, usually due to couplings of about 7 Hz, are not resolved (23). The average linewidth we obtain is about 30 Hz. Because of this, fitting in-vivo signals with the high resolution multiplet structures as prior knowledge, gives large estimation errors and is not useful. Therefore, the number of sinusoids used to fit each signal component was taken as small as possible, but large enough to quantify the signal sufficiently accurately. The number of sinusoids used to fit chemically distinct proton signals, and the results of the fits are given in Table 1. The Cramér Rao errors for the frequencies are much smaller than 0.1 Hz and therefore not given in Table 1. Because several peaks severely overlap, the associated damping constants are difficult to estimate. Therefore the damping constants were estimated with the peakpick method and kept fixed. No errors are given for these parameters. De Graaf et al (21) described that after division of the experimental NMR time domain signal by a reference signal, pure Lorentzian line shapes are obtained. Due to this division however, the damping constant of component j of the corrected signal under ideal circumstances is \( \left( T_2^{-1} \right)_\text{cor} = T_2^{-1} - T_2^{-1} \). This effect of the reference signal is eliminated if Lorentzian windowing is applied on the corrected signal with the transverse relaxation time of the reference signal \( T_2^{\text{ref}} \). It must be noted that the fitted sinusoids in fact consist of a large number of signal components which are not resolved. Therefore, the given damping constants can not be interpreted as true line widths. In Figure 4 the FFT spectra of the signals and the fits
are shown. A time domain window is only used for the FFT, after the fitting process. The signal intensities are affected by the J-modulation and the water suppression pulse (21). These effects can be taken into account by an effective intensity value $I_{E_k}$. This value, defined in Eq. [10], is in Table 1 given for the considered metabolites.

$$I_{E_k} = \left( \frac{A_k}{A_{NAA}} \right) \left( \frac{C_{NAA}}{C_k} \right) \left( \frac{3}{N_k} \right) \quad [10]$$

with $C_k =$ concentration of the compound corresponding to component $k$,

$C_{NAA} =$ concentration of NAA,

$A_k =$ amplitude of component $k$,

$A_{NAA} =$ amplitude of the NAA $\text{CH}_3$ component,

$N_k =$ number of protons contributing to component $k$.

Note that the definition of $I_{E_k}$ implies that for the NAA $\text{CH}_3$ component this value equals 1. This definition is chosen in this way because these $\text{CH}_3$ protons are not coupled and the interpulse time $\delta$ is chosen to detect this component optimally as described before.

From the results of the separate solutions prior knowledge was extracted. For each metabolite the ratios of the amplitudes of the sinusoids were calculated. If the concentration of a metabolite changes, these ratios remain constant. Using this as prior knowledge, only one amplitude for every metabolite need be fitted. In previous
Table 1  Fitting results (after applying QUALITY and Lorentzian damping, see section 4) of the separate solutions of 40 mM NAA, 40 mM GLU, 40 mM GLN, and 20 mM ASP. The definition of $I_{E\kappa}$ is given in Eq. [10].

<table>
<thead>
<tr>
<th></th>
<th>amplitude</th>
<th>error(2σ)</th>
<th>damping</th>
<th>frequency</th>
<th>concentration</th>
<th>$I_{E\kappa}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a.u.)</td>
<td>(%)</td>
<td>(ms)</td>
<td>(Hz)</td>
<td>(mM)</td>
<td></td>
</tr>
<tr>
<td>NAA CH$_3$</td>
<td>12468</td>
<td>0.1</td>
<td>25.0</td>
<td>837.6</td>
<td>40 ± 1</td>
<td>1.000</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>1557</td>
<td>0.1</td>
<td>20.0</td>
<td>703.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta'_1$</td>
<td>1857</td>
<td>0.1</td>
<td>20.0</td>
<td>686.9</td>
<td></td>
<td>0.822</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>1990</td>
<td>0.1</td>
<td>20.0</td>
<td>642.4</td>
<td></td>
<td>0.825</td>
</tr>
<tr>
<td>$\beta'_2$</td>
<td>1440</td>
<td>0.1</td>
<td>20.0</td>
<td>627.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASP $\beta_2$</td>
<td>250</td>
<td>-</td>
<td>25.0</td>
<td>568.0</td>
<td>20.0 ± .5</td>
<td></td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>680</td>
<td>-</td>
<td>13.0</td>
<td>633.2</td>
<td></td>
<td>0.37</td>
</tr>
<tr>
<td>$\beta'_1$</td>
<td>642</td>
<td>-</td>
<td>18.0</td>
<td>601.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLN $\gamma$</td>
<td>1992</td>
<td>0.5</td>
<td>15.0</td>
<td>712.1</td>
<td>40 ± 1</td>
<td>0.240</td>
</tr>
<tr>
<td>$\beta$</td>
<td>738</td>
<td>0.5</td>
<td>22.0</td>
<td>804.5</td>
<td></td>
<td>0.089</td>
</tr>
<tr>
<td>GLU $\gamma$</td>
<td>3693</td>
<td>0.3</td>
<td>18.0</td>
<td>737.8</td>
<td>40 ± 1</td>
<td>0.444</td>
</tr>
<tr>
<td>$\beta$</td>
<td>979</td>
<td>0.3</td>
<td>15.0</td>
<td>820.5</td>
<td></td>
<td>0.157</td>
</tr>
<tr>
<td>$\beta'$</td>
<td>321</td>
<td>0.3</td>
<td>18.0</td>
<td>800.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
studies (4) we found that the frequency differences of the sinusoids of these metabolites do not change. Therefore only one frequency has to be fitted for the entire signal. It is important to note that the frequency dependent distortion of the signal intensity due to the water suppression pulse and the effects of J-modulation are taken into account in the method described above. The only restriction is that all measurements must be performed under the same experimental circumstances.

Note that because of QUALITY and because of the suppression of the FID after the refocusing pulse, the forward and backward damping constants are equal.

4.2 USING PRIOR KNOWLEDGE IN-VITRO

To test the program while using prior knowledge, a mixture of the four metabolites was solved in water with known concentrations. Again the solution was buffered to pH 7. With the sequence of Figure 3 (using the rat brain phantom and the surface coil), a water suppressed echo was measured. The signal was corrected with the QUALITY method and after that, Lorentzian time domain windowing (50 ms) was applied. Finally the signal was fitted using prior knowledge as described in 4.1. In addition, all damping factors were fixed to the values obtained from the fits of the separate solutions, see Table 1. The FFT spectra of the measured signal and the fit are shown in Figure 5. A
Table 2 Fitting results of the mixture of 10 mM NAA, 10 mM GLU, 10 mM GLN, and 5 mM ASP. The relative fitted concentrations are calculated with $I_{\text{EKE}}$ of Table 1. This value is for NAA set to 1.

<table>
<thead>
<tr>
<th>metabolite</th>
<th>amplitude (a.u.)</th>
<th>error (2(\sigma)) (%)</th>
<th>true concentrations (mM)</th>
<th>relative fitted concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA $\text{CH}_3$</td>
<td>3064</td>
<td>0.3</td>
<td>$10.0 \pm 0.3$</td>
<td>1.0</td>
</tr>
<tr>
<td>ASP $\beta^a$</td>
<td>373</td>
<td>4</td>
<td>$5.0 \pm 0.2$</td>
<td>$0.49 \pm 0.04$</td>
</tr>
<tr>
<td>GLN $\gamma$</td>
<td>502</td>
<td>2</td>
<td>$10.0 \pm 0.3$</td>
<td>$1.02 \pm 0.05$</td>
</tr>
<tr>
<td>GLU $\gamma$</td>
<td>935</td>
<td>1</td>
<td>$10.0 \pm 0.3$</td>
<td>$1.03 \pm 0.04$</td>
</tr>
</tbody>
</table>

$^a$ values are given for the sum of the $\beta$ resonances.

time domain window is only used for the FFT, after the fitting process. Using the $I_{\text{EKE}}$ values of table 1, relative concentrations of the four metabolites in the mixture can be determined from the fitting results, see table 2. For NAA this value is taken 1. Note that the fitted results agree with the true values.

In principle it is possible to obtain absolute metabolite concentrations. In that case corrections for relaxation have to be taken into account (4).
Figure 5. The spectral region between 1.5 and 3 ppm of the experimental (solid line) and fitted (dotted line) signal of the mixture with 10 mM NAA, 5 mM ASP, 10 mM GLN, and 10 mM GLU. Gaussian time domain windowing was applied with 21 ms before the FFT as is done with in-vivo measurements.

4.3 ADAPTATIONS OF PRIOR KNOWLEDGE FOR IN-VIVO USE

The prior knowledge obtained from the in-vitro measurements is used to fit a series of in-vivo measurements. The fitting strategy was the same as described in subsection 4.2.
Besides the signal components of the metabolites ASP, GLN, GLU and NAA for which prior knowledge is available, components of choline (CHO)
Table 3 Prior knowledge used to fit the in-vivo measurements (after applying QUALITY and Lorentzian windowing).

<table>
<thead>
<tr>
<th></th>
<th>amplitude ratios (a.u.)</th>
<th>damping (ms)</th>
<th>frequency difference (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA CH₃</td>
<td>1.0</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>β₁</td>
<td>0.1249</td>
<td>20.0</td>
<td>134.0</td>
</tr>
<tr>
<td>β₁'</td>
<td>0.1489</td>
<td>20.0</td>
<td>150.7</td>
</tr>
<tr>
<td>β₂</td>
<td>0.1600</td>
<td>20.0</td>
<td>191.2</td>
</tr>
<tr>
<td>β₂'</td>
<td>0.1155</td>
<td>20.0</td>
<td>206.0</td>
</tr>
<tr>
<td>ASP β₂</td>
<td>1.0</td>
<td>25.0</td>
<td>179.6</td>
</tr>
<tr>
<td>β₁</td>
<td>2.7230</td>
<td>13.0</td>
<td>204.4</td>
</tr>
<tr>
<td>β₁'</td>
<td>2.5700</td>
<td>18.0</td>
<td>231.6</td>
</tr>
<tr>
<td>GLN γ</td>
<td></td>
<td>15.0</td>
<td>129.5</td>
</tr>
<tr>
<td>β</td>
<td></td>
<td>22.0</td>
<td>33.1</td>
</tr>
<tr>
<td>GLU γ</td>
<td></td>
<td>18.0</td>
<td>99.8</td>
</tr>
<tr>
<td>β₁</td>
<td></td>
<td>15.0</td>
<td>17.1</td>
</tr>
<tr>
<td>β₂</td>
<td></td>
<td>18.0</td>
<td>37.1</td>
</tr>
<tr>
<td>CHO</td>
<td></td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>CRE</td>
<td></td>
<td>25.0</td>
<td></td>
</tr>
</tbody>
</table>

and (phospho) creatine (PCR) can be recognized. The latter metabolites give in the spectrum peaks at 3.2 and 3.0 ppm respectively. These signal components can each be quantified by a single damped sinusoid.
The damping constants of these sinusoids are taken equal to the damping constants of the NAA-CH$_3$ signal component. The resonance frequencies and the amplitudes of CHO and PCR are estimated by the fitting program without further constraints.

In-vivo, it is difficult to quantify the $\beta$ signal components of GLU and GLN because these are very small. Moreover, in the spectrum these peaks are severely overlapped by the relatively high NAA-CH$_3$ peak and some small resonances of other metabolites, which are present in low concentrations (24). Due to phase problems which therefore occur in the in-vivo measurements, the NAA-CH$_3$ peak influences the estimated intensities of the GLU and GLN $\beta$ peaks. Since the $\gamma$ and $\beta$ intensities of GLU and GLN are by the prior knowledge related with each other, the $\gamma$ amplitudes are not correctly estimated as well. To avoid this problem, the amplitude relations between the $\gamma$ and $\beta$ peaks of GLU and GLN are not used in the fit of the in-vivo measurements. The GLU and GLN intensities are determined from the estimations of the amplitudes of the $\gamma$ signal components although these are overlapped by the $\beta_1$ and $\beta_1'$ resonances of NAA. The latter can be determined because they are related with the amplitude of the NAA-CH$_3$ resonance which can be fitted rather accurately.

For the in-vivo measurements some fixed resonance frequencies had to be slightly changed as compared to the in-vitro situation. These shifts might be explained by the difference in the chemical environment between the in-vitro and the in-vivo situation. The $\beta_1$ and $\beta_1'$ resonance frequencies of NAA were shifted 4 Hz (-0.013 ppm), the $\gamma$
resonance frequency of GLN was shifted -3 Hz (0.01 ppm). With these adaptations a series of in-vivo measurements could satisfactorily be fitted. The prior knowledge used to quantify the in-vivo measurements is given in Table 3. The damping constants are obtained from Table 1.

4.4 RESULTS OF THE IN-VIVO MEASUREMENTS

A complete series of eleven measurements was quantified. Before the fitting procedure was started the line shape correction method QUALITY and Lorentzian windowing (50 msec) were applied for reasons described before. The average number of iterations used by the fitting program is 10. Starting values are obtained from the in-vitro results. The iteration process is stopped if the estimates do not change more than $10^{-4}$ %. As an illustrative example, in Figure 6, the spectra are shown of an in-vivo signal and its fit. The signal was measured seven hours after inducing liver ischemia and one hour after administration of a partial inverse benzodiazepine receptor agonist. The spectrum of the reconstructed signal coincides reasonably well with the experimental spectrum in the frequency region between 1.9 and 3.3 ppm. Outside this region the spectra differ because no signal components are fitted. In Table 4 the fitted parameters are given for this signal. Note that the errors of the estimated amplitudes of the $\beta$ peaks of GLU and GLN are high. This is probably due to the strong overlap as mentioned before. The $\gamma$ resonance intensities are fitted more accurately.
Table 4 Fitting results of the in-vivo measurement. Errors are Cramer Rao lower bounds (2 \* \( \sigma \)). 1 ppm = 300 Hz. The frequency errors are smaller than 0.01 Hz and omitted.

<table>
<thead>
<tr>
<th></th>
<th>amplitude (a.u.)</th>
<th>error (%)</th>
<th>damping (ms)</th>
<th>frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA CH(_3)</td>
<td>32444</td>
<td>1.2</td>
<td>25.0</td>
<td>796.8</td>
</tr>
<tr>
<td>(\beta)</td>
<td>4052</td>
<td>1.2</td>
<td>20.0</td>
<td>662.8</td>
</tr>
<tr>
<td>(\beta')</td>
<td>4831</td>
<td>1.2</td>
<td>20.0</td>
<td>646.1</td>
</tr>
<tr>
<td>(\beta_2)</td>
<td>5191</td>
<td>1.2</td>
<td>20.0</td>
<td>605.6</td>
</tr>
<tr>
<td>(\beta'_2)</td>
<td>3747</td>
<td>1.2</td>
<td>20.0</td>
<td>590.8</td>
</tr>
<tr>
<td>ASP (\beta_2)</td>
<td>349</td>
<td>25</td>
<td>25.0</td>
<td>617.2</td>
</tr>
<tr>
<td>(\beta_1)</td>
<td>951</td>
<td>25</td>
<td>13.0</td>
<td>592.4</td>
</tr>
<tr>
<td>(\beta'_1)</td>
<td>898</td>
<td>25</td>
<td>18.0</td>
<td>565.2</td>
</tr>
<tr>
<td>GLN (\gamma)</td>
<td>5228</td>
<td>7.6</td>
<td>15.0</td>
<td>667.3</td>
</tr>
<tr>
<td>(\beta)</td>
<td>5234</td>
<td>9.5</td>
<td>22.0</td>
<td>763.7</td>
</tr>
<tr>
<td>GLU (\gamma)</td>
<td>9107</td>
<td>4.4</td>
<td>18.0</td>
<td>697.0</td>
</tr>
<tr>
<td>(\beta_1)</td>
<td>1325</td>
<td>45</td>
<td>15.0</td>
<td>779.7</td>
</tr>
<tr>
<td>(\beta_2)</td>
<td>434</td>
<td>45</td>
<td>18.0</td>
<td>759.7</td>
</tr>
<tr>
<td>CHO</td>
<td>9523</td>
<td>3.2</td>
<td>25.0</td>
<td>437.1</td>
</tr>
<tr>
<td>CRE</td>
<td>23430</td>
<td>1.3</td>
<td>25.0</td>
<td>491.0</td>
</tr>
</tbody>
</table>
Figure 6. The spectral range between 1.3 and 3.5 ppm of the experimental (solid line) and fitted (dotted line) in-vivo signal. Only signal components between 1.9 and 3.3 ppm are fitted. Assignments are given in Figure 4. Additionally, at 3.2 ppm Choline and at 3.0 (Phospho) Creatine peaks are visible.

4.5 DISCUSSION.

Some remarks must be given concerning the interpretation of the fitting results.

1) The model function for each line is an exponentially damped sinusoid. The measured signal however can only be described by this
model function if the applied line shape correction method, QUALITY, really yields perfect Lorentzian line shapes. If this is not the fact, systematic errors will occur.

2) Unknown background resonances might be present. In the frequency region between 1.9 and 3.3 ppm the most important contributions could be expected from γ-amino butyric acid (GABA) which has a concentration in the millimolar range (25). However, we have never noticed any contributions from GABA in our spectra and therefore this compound is not taken into account in the fitting procedure.

3) Another source of systematic errors arises from ignoring signals which have resonance frequencies outside the frequency region of interest as e.g. the not completely suppressed water signal. It can be shown that these errors are proportional to the amount of overlap in the spectrum of the fitted and the not-fitted components (26). If this overlap is very small as in Figures 5 and 6, these systematic errors can be ignored.

4) If the used prior knowledge is not appropriate, systematic errors will occur in the estimations of the parameters.

5) Motions of the animal give distortions in the signal and errors in the subsequent quantification which are not corrected by the QUALITY method, see for more details (23).

6) Differences in transverse relaxation times will affect the amplitude ratios and the damping constants. A thorough discussion of these errors is given in (4).

The most important source of errors is mentioned at 5).
Despite these remarks, from the complete series of in-vivo measurements it can be concluded that the fitting procedure, in combination with prior knowledge is able to satisfactorily fit the in-vivo signals.

5. CONCLUSIONS.

A time domain Gauss-Newton fitting procedure is described, which is able to fit echo-like signals as well as normal FID's. Damping constants of the first and second part of the echo do not have to be identical. The computing time is considerably reduced because part of the computations are analytically worked out in closed-form. Prior knowledge is incorporated to diminish the number of parameters to be fitted and to improve accuracy.

Actual prior knowledge is obtained from model solutions of aspartate, glutamine, glutamate and N-acetyl aspartate. This knowledge is used to fit a mixture of these metabolites. The results of this fit are in agreement with the true concentrations. With some minor changes of the prior knowledge as obtained in-vitro, the program was able to fit a series of in-vivo measurements.
APPENDIX 1. PRIOR KNOWLEDGE ABOUT FREQUENCIES

Here we consider adaptations of the model function equal to $\delta \tilde{x}$ as a result of a change $\delta \tilde{\omega}$ in $\tilde{\omega}$. Since only the frequency part of Eq. [7] is considered, distinction between the $\xi$ and $\zeta$ part is not necessary and therefore omitted. Three kinds of prior knowledge concerning frequencies are distinguished:

1) A difference between two frequencies is known: $\omega_{\ell} = \omega_k + a$, $\Rightarrow \delta \omega_{\ell} = \delta \omega_k$ with $k < \ell$ and $a$ known. The change of the signal as a result of small changes $\delta \omega_m$, $m = 1, \ldots, K$ can be expressed as:

$$
\delta \begin{pmatrix}
\tilde{x}_{-N} \\
\vdots \\
\tilde{x}_M
\end{pmatrix} = \begin{pmatrix}
-i \text{NF}_{-N,1} & \ldots & -i \text{NF}_{-N,k} & \ldots & -i \text{NF}_{-N,\ell} & \ldots & -i \text{NF}_{-N,K} \\
\vdots & & \vdots & & \vdots & & \vdots \\
i \text{MF}_{M,1} & \ldots & i \text{MF}_{M,k} & \ldots & i \text{MF}_{M,\ell} & \ldots & i \text{MF}_{M,K}
\end{pmatrix} \begin{pmatrix}
\delta \omega_{1,c_1} \\
\vdots \\
\delta \omega_{K,c_K}
\end{pmatrix}
$$

$$
\begin{pmatrix}
-i \text{NF}_{-N,1} & \ldots & -i \text{NF}_{-N,k} & \ldots & -i \text{NF}_{-N,\ell} & \ldots & -i \text{NF}_{-N,K} \\
\vdots & & \vdots & & \vdots & & \vdots \\
i \text{MF}_{M,1} & \ldots & i \text{MF}_{M,k} & \ldots & i \text{MF}_{M,\ell} & \ldots & i \text{MF}_{M,K}
\end{pmatrix} \begin{pmatrix}
\delta \omega_{1,c_1} \\
\vdots \\
\delta \omega_{K,c_K}
\end{pmatrix}
$$
\[
\begin{bmatrix}
-i\eta_{N,1} & \cdots & -i\eta_{N,K} & \cdots & -i\eta_{N,1} & \cdots & -i\eta_{N,K} \\
\vdots & & \vdots & & \vdots & & \vdots \\
i\eta_{M,1} & \cdots & i\eta_{M,K} & \cdots & i\eta_{M,1} & \cdots & i\eta_{M,K}
\end{bmatrix}
\begin{bmatrix}
\delta\omega_{1}c_{1} \\
\delta\omega_{1}c_{K} \\
\delta\omega_{N}c_{K} \\
\delta\omega_{N}c_{K}
\end{bmatrix}
\]

\[
\begin{bmatrix}
-i\eta_{N,1} & \cdots & -i\eta_{N,K} & \cdots & -i\eta_{N,1} & \cdots & -i\eta_{N,K} \\
\vdots & & \vdots & & \vdots & & \vdots \\
i\eta_{M,1} & \cdots & i\eta_{M,K} & \cdots & i\eta_{M,1} & \cdots & i\eta_{M,K}
\end{bmatrix}
\begin{bmatrix}
\delta\omega_{1}c_{1} \\
\delta\omega_{1}c_{K} \\
\delta\omega_{N}c_{K} \\
\delta\omega_{N}c_{K}
\end{bmatrix}
\]

\[
\begin{bmatrix}
-i\eta_{N,1} & \cdots & -i\eta_{N,K} & \cdots & -i\eta_{N,1} & \cdots & -i\eta_{N,K} \\
\vdots & & \vdots & & \vdots & & \vdots \\
i\eta_{M,1} & \cdots & i\eta_{M,K} & \cdots & i\eta_{M,1} & \cdots & i\eta_{M,K}
\end{bmatrix}
\begin{bmatrix}
\delta\omega_{1}c_{1} \\
\delta\omega_{1}c_{K} \\
\delta\omega_{N}c_{K} \\
\delta\omega_{N}c_{K}
\end{bmatrix}
\]

\[
\Rightarrow \delta x = \overline{F}_{R}^{-1} p_{R}
\]

Frequency $\omega_{\ell}$ is not calculated during the iterative process but afterwards. Because of this, columns $\ell$ and $K$ of $\overline{F}$ can be concatenated and row $\ell$ of $p$ can be eliminated.

2) If a frequency is known, $\omega_{\ell} = \alpha$, it follows that $\delta\omega_{\ell} = 0$. Therefore element $\ell$ from $\overline{p}$ and column $\ell$ from $\overline{F}$ can be eliminated.
\[ \delta \begin{pmatrix} x_{-N} \\ \vdots \\ x_M \end{pmatrix} = \begin{pmatrix} -iNF_{-N,1} & \ldots & -iNF_{-N,K} \\ \vdots & & \vdots \\ iNF_{M,1} & \ldots & iNF_{M,K} \end{pmatrix} \begin{pmatrix} \delta \omega_1 c_1 \\ \vdots \\ \delta \omega_K c_K \end{pmatrix} \]

\[ \Rightarrow \delta x = \vec{p}_R \vec{p}_R \]

3) If multiplet structures are known, besides amplitude relations also frequency relations can be used to diminish the number of parameters to be fitted. This is illustrated by a specific example because the general formulae get quite large and are difficult to interpret.

A triplet and a doublet are considered with equal but unknown coupling constant \( \Delta \omega \). The frequencies are thus given by

\[ \omega_1, \omega_2 = \omega_1 + \Delta \omega, \omega_3 = \omega_1 + 2\Delta \omega, \omega_4, \omega_5 = \omega_4 + \Delta \omega \]

\[ \delta \omega_2 = \delta \omega_1 + \delta \Delta \omega, \delta \omega_3 = 2 \delta \omega_1 + 2 \delta \Delta \omega, \delta \omega_5 = \delta \omega_4 + \delta \Delta \omega. \]

Only the frequency relations are treated here, so \( \vec{p} \) is given by

\[ \vec{p} = \begin{pmatrix} \delta \omega_1 c_1 \\ \delta \omega_2 c_2 \\ \delta \omega_3 c_3 \\ \delta \omega_4 c_4 \\ \delta \omega_5 c_5 \end{pmatrix} = \begin{pmatrix} \delta \omega_1 c_1 \\ (\delta \omega_1 + \delta \Delta \omega)c_2 \\ (\delta \omega_1 + 2\delta \Delta \omega)c_3 \\ \delta \omega_4 c_4 \\ (\delta \omega_4 + \delta \Delta \omega)c_5 \end{pmatrix} = \begin{pmatrix} \delta \omega_1 c_1 \\ \delta \omega_1 c_1(c_2/c_1) + \delta \Delta \omega c_2 \\ \delta \omega_1 c_1(c_3/c_1) + 2 \delta \Delta \omega c_2(c_3/c_2) \\ \delta \omega_4 c_4 \\ \delta \omega_4 c_4(c_5/c_4) + \delta \Delta \omega c_2(c_5/c_2) \end{pmatrix} \]

Using the last result, the original problem can be reduced as follows.
All terms with $\delta \omega_1$ of the first three rows of $\vec{p}$ are taken together in the first row. All terms with $\delta \Delta \omega$ are taken together in the second row of $\vec{p}$. The terms with $\delta \omega_4$ are combined in the third row. The original rows 3 and 5 of $\vec{p}$ and columns 3 and 5 of $\vec{F}$ are eliminated.

$$
\left( \begin{array}{c}
\omega^\prime \mathbf{N} \\
\vdots \\
\omega^\prime \mathbf{M}
\end{array} \right) =
\left( \begin{array}{ccccc}
-i \mathbf{N}, 1 & -i \mathbf{N}, 2 & -i \mathbf{N}, 3 & -i \mathbf{N}, 4 & -i \mathbf{N}, 5 \\
\vdots & \vdots & \vdots & \vdots & \\
\imath \mathbf{M}, 1 & \imath \mathbf{M}, 2 & \imath \mathbf{M}, 3 & \imath \mathbf{M}, 4 & \imath \mathbf{M}, 5
\end{array} \right)
\left( \begin{array}{c}
\delta \omega_1 c_1 \\
\delta \omega_2 c_2 \\
\delta \omega_3 c_3 \\
\delta \omega_4 c_4 \\
\delta \omega_5 c_5
\end{array} \right)
$$

$$
\vec{F}_R = \vec{p}_R
$$

$$
\vec{F}_R =
\left( \begin{array}{c}
-i \mathbf{N} \left( \mathbf{F}_N, 1^+ \left( c_2/c_1 \right) \mathbf{F}_N, 2 + (c_3/c_1) \mathbf{F}_N, 3 \right) \\
\vdots \\
\imath \mathbf{M} \left( \mathbf{F}_M, 1^+ \left( c_2/c_1 \right) \mathbf{F}_M, 2 + (c_3/c_1) \mathbf{F}_M, 3 \right)
\end{array} \right)
$$

$$
\left( \begin{array}{c}
-i \mathbf{N} \left( \mathbf{F}_N, 2^+ 2 \left( c_3/c_2 \right) \mathbf{F}_N, 3^+ \left( c_5/c_2 \right) \mathbf{F}_N, 5 \right) \\
\vdots \\
\imath \mathbf{M} \left( \mathbf{F}_M, 2^+ 2 \left( c_3/c_2 \right) \mathbf{F}_M, 3 + \left( c_5/c_2 \right) \mathbf{F}_M, 5 \right)
\end{array} \right)
$$

$$
\vec{p}_R =
\left( \begin{array}{c}
\delta \omega_1 c_1 \\
\delta \Delta \omega c_2 \\
\delta \omega_4 c_4
\end{array} \right)
$$

138
Of course, if yet another multiplet exists with the same $\Delta \omega$, or if the triplet or doublet are replaced by larger multiplets, the same kind of reduction formulae can be derived. In addition, amplitude relations occurring in the multiplets can be handled concurrently as described in 3.1.
APPENDIX 2. PRIOR KNOWLEDGE ABOUT DAMPING CONSTANTS

In this appendix only the damping part of Eq. [7] is worked out. Several kinds of prior knowledge concerning damping constants can be considered. Three of these are treated below.

1) The difference between two backward damping constants or two forward damping constants is known: \( \xi_{\ell} = \xi_{k} + a \Rightarrow \delta \xi_{\ell} = \delta \xi_{k} \) or \( \zeta_{\ell} = \zeta_{k} + a \Rightarrow \delta \zeta_{\ell} = \delta \zeta_{k} \).

In this case the same relations can be derived as was done in appendix 1 for a known difference between two frequencies.

2) A ratio of two damping constants is known: \( \xi_{\ell} = a \xi_{k} \) or \( \zeta_{\ell} = a \zeta_{k} \). This means \( \delta \xi_{\ell} = a \delta \xi_{k} \) or \( \delta \zeta_{\ell} = a \delta \zeta_{k} \). The same reduction formulae as at 1) can be derived, only the ratio \( c_{\ell}/c_{k} \) must be replaced by: \( a c_{\ell}/c_{k} \).

3) If a difference between a forward and a backward damping constant is known: \( \zeta_{\ell} = \xi_{k} + a \) the reduction formulae are more complicated. The change of the signal as a result of a change of the damping factors is:

140
\[
\begin{pmatrix}
\delta x_0 \\
\delta x_1 \\
\vdots \\
\delta x_M
\end{pmatrix} =
\begin{pmatrix}
-N_{\text{N},1} & \cdots & -N_{\text{N},K} & 0 & \cdots & 0 & 0 \\
\vdots & \ddots & \vdots & \ddots & \ddots & \vdots & \vdots \\
-F_{1,1} & \cdots & -F_{1,K} & 0 & \cdots & 0 & 0 \\
0 & \cdots & 0 & 0 & \cdots & 0 & 0
\end{pmatrix}
\begin{pmatrix}
\delta \xi_1 c_1 \\
\vdots \\
\delta \xi_K c_K \\
\delta \xi_1 c_{1/l} \\
\vdots \\
\delta \xi_K c_{1/K}
\end{pmatrix}
\]

Using \( \delta \xi_{1/l} = \delta \xi_K \), it follows that

\[
\begin{pmatrix}
-N_{\text{N},1} & \cdots & -N_{\text{N},K} & 0 & \cdots & 0 & 0 \\
\vdots & \ddots & \vdots & \ddots & \ddots & \vdots & \vdots \\
-F_{1,1} & \cdots & -F_{1,K} & 0 & \cdots & 0 & 0 \\
0 & \cdots & 0 & 0 & \cdots & 0 & 0
\end{pmatrix}
\begin{pmatrix}
\delta \xi_1 c_1 \\
\vdots \\
\delta \xi_K c_K \\
\delta \xi_1 c_{1/l} \\
\vdots \\
\delta \xi_K c_{1/K}
\end{pmatrix}
\]

The two rows of \( \bar{p} \) with \( \delta \xi_K c_K \) can be combined:
\[
\begin{pmatrix}
-N^F_{-N,1} & \ldots & -N^F_{-N,K} & \ldots & -N^F_{N,K} & 0 & \ldots & 0 \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\
-N^F_{-1,1} & \ldots & -N^F_{-1,K} & \ldots & -F^F_{-1,K} & 0 & \ldots & 0 \\
0 & \ldots & 0 & \ldots & 0 & 0 & \ldots & 0 \\
0 & \ldots & (c_\ell/c_{k})F^F_{1,\ell} & \ldots & 0 & F_{1,1} & \ldots & F_{1,K} \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\
0 & \ldots & (c_\ell/c_{k})F^M_{M,\ell} & \ldots & 0 & F^M_{M,1} & \ldots & F^M_{M,K}
\end{pmatrix}
= 
\begin{pmatrix}
\delta \xi_1 c_1 \\
\vdots \\
\delta \xi_K c_K \\
\delta \zeta_1 c_1 \\
\vdots \\
\delta \zeta_K c_K
\end{pmatrix}
\]

\[\Rightarrow \delta \tilde{x} = \bar{F} \bar{p}
\]

In conclusion, column \( \ell \) of the backward part of \( \bar{F} \) is added to column \( k \) of the forward part of \( \bar{F} \) after multiplication with \( c_\ell/c_{k} \). Hereafter, element \( \ell \) of the backward part of \( \bar{p} \) and column \( \ell \) of the backward part of \( \bar{F} \) are eliminated.
REFERENCES

8) D.S. Stephenson, Prog. NMR Spectrosc. 20, 515 (1988), and references therein.
24) C. Arus, Yen-Chang, M. Barany, Physiological Chemistry and Physics and Medical NMR 17, 23 (1985)
CHAPTER 7. SUMMARY AND CONCLUSIONS.

1. GENERAL

Due to the fact that with proton MRS signals are detected from many metabolites whose spectral components severely overlap, it is difficult to assign these metabolites and to quantify their concentrations. In this thesis two possible solutions for this problem are considered. The first one is measuring selectively signals from only specific metabolites with especially multiple quantum editing techniques. The second solution is the use of a sophisticated data processing technique which applies as much prior knowledge as possible to analyze the measured signals.

If data processing techniques are able to differentiate between the signal components of the metabolites, non-editing sequences which give signals from all metabolites are favorite. However, in some cases editing techniques are the only choice (Chapter 1).

2. MULTIPLE QUANTUM EDITING TECHNIQUES

Several important aspects of multiple quantum editing techniques are investigated. To prevent motion artifacts as much as possible, one or at most two scan sequences must be used. Zero and double quantum sequences can yield a good metabolite selectivity but give at most 50\% of the maximal signal intensity. A sequence that combines the zero and double quantum coherences yields 100\% of the maximal signal intensity but needs a phase cycling scheme to adequately suppress water and lipid signals. Phase cycling schemes are not attractive because motion artifacts can occur. The effects of static field inhomogeneity on the multiple quantum coherences can be corrected for by shifting the last 180 pulse in time. Inhomogeneity of the RF field effects the investigated multiple quantum sequences in about the same way and decreases the volume from which signals are detected.
Extensions of multiple quantum sequences using the signal modulation corresponding to the multiple quantum frequencies, are given to obtain improved editing capabilities. (Chapter 2).

It is shown that the multiple quantum sequences can be combined with localization techniques. As an example, results of in-vivo lactate measurements with a localized zero quantum sequence are described. (Chapter 3).

In in-vitro studies it is shown that multiple quantum editing techniques can be used to measure the $\gamma$ glutamine peak without overlap of the $\gamma$ glutamate peak and vice versa. A double quantum sequence is optimized for this purpose with respect to selectivity and signal intensity. The measured signal intensity is lower as compared to the intensity obtained with a spin echo experiment and hardly acceptable for in-vivo applications. An alternative editing method based on spin echo sequences is described. This method gives larger signal intensities but is sensitive to subtraction errors that can occur due to motion- and relaxation effects (Chapter 4).

As an application of the developed techniques, an in-vivo study was performed in collaboration with the Academic Medical Center of Amsterdam, The Netherlands. O’Connor et al found a protective effect of L-carnitine on acute hyperammonemic encephalopathy in mice. This effect is investigated in rats by means of proton MRS and clinical parameters. A spin echo sequence with two dimensional localization was used to measure the normal water suppressed in-vivo spectra. Additionally, a double quantum editing sequence was used to study changes in the lactate concentration. Although a small protective effect was found in the EEG parameters, the striking effect of L-carnitine against the toxicity of hyperammonemia as shown by O’Connor et al has not been confirmed in our experiments. The double quantum method performed well (Chapter 5).
3. DATA PROCESSING TECHNIQUES

The possibility of using prior knowledge is incorporated in a fast and flexible iterative time domain fitting procedure. This fitting procedure can be used to analyze free induction decays as well as echo-like signals. The model function is an exponentially damped sinusoid. Calculations are analytically worked out to reduce the computing time. From measurements of model solutions prior knowledge is extracted. Due to the application of prior knowledge, the accuracy is improved. A signal consisting of several components which in the frequency domain severely overlap, is quantified with the fitting procedure.

With some minor changes of the described prior knowledge, the same procedure is used to fit echo-like signals obtained from in-vivo measurements of the rat brain, measured with a water suppressed spin echo sequence (Chapter 6).
SAMENVATTING EN CONCLUSIES.

Titel: In-vivo Proton Magnetische Resonantie Spectroscopie: Evaluatie van Meer-Quantum Technieken voor Spectrum Editing en een Tijd Domein Fitting Procedure voor Quantificatie.

1. ALGEMEEN

Met proton magnetische resonantie spectroscopie (MRS) worden signalen gemeten van metabolieten waarvan de frequentiecomponenten elkaar sterk overlappen. Door die overlap is het moeilijk de verschillende signaalcomponenten toe te kennen aan de metabolieten en de concentraties te bepalen. In dit proefschrift worden twee oplossingen voor dit probleem uiteengezet. De eerste is het selectief meten van signalen van slechts een beperkt aantal metabolieten met in de hoofdzaak meer-quantum technieken. De tweede oplossing is het gebruik van een data-verwerkingstechniek die zo veel mogelijk voorkennis gebruikt bij het analyseren van de gemeten signalen.
Als met data-verwerkingstechnieken de verschillende signaalcomponenten van de metabolieten te onderscheiden zijn, dan verdienen niet-selectieve meettechnieken de voorkeur. In sommige gevallen is er geen keus mogelijk en moeten selectieve meettechnieken gebruikt worden (hoofdstuk 1).

2. SELECTIEVE MEER-QUANTUM TECHNIEKEN

Een aantal belangrijke aspecten van selectieve meer-quantum technieken zijn onderzocht. Om verstoringen te voorkomen die veroorzaakt worden door bewegingen, moeten technieken gebruikt worden die één of ten hoogste twee pulsreeksen nodig hebben. Met nul- en twee-quantum technieken kan een goede metabolietselectiviteit verkregen worden maar ze geven ten hoogste 50 % van de maximale signaalintensiteit. Een methode waarmee de nul- en twee-quantum signalen tegelijkertijd
gemeten worden, levert 100 % van de signaalintensiteit. Deze methode geeft echter alleen een goede onderdrukking van de vet- en watersignalen als een fasecyclus gebruikt wordt. Fasecycli zijn in-vivo niet gewenst omdat ze verstoringen kunnen geven in de meetresultaten als er bewegingen zijn geweest. Er kan gecorrigeerd worden voor de effecten die inhomogeniteiten van het statische magneetveld hebben op de meetresultaten. Dit moet gebeuren door de laatste 180° puls te verschuiven ten opzichte van de laatste 90° puls. Er zijn uitbreidingen van de meer-quantum technieken gegeven die gebruik maken van de modulatie, veroorzaakt door de meer-quantum frequenties, waarmee een betere selectiviteit verkregen wordt (hoofdstuk 2).

De meer-quantum technieken kunnen gecombineerd worden met localisatie technieken. Als voorbeeld is beschreven hoe lactaat (melkzuur) in-vivo gemeten kan worden met een gelocaliseerde nul-quantum techniek (Hoofdstuk 3).

Meer-quantum technieken kunnen gebruikt worden om de γ-piek van glutamaat te meten zonder overlap van de γ-piek van glutamine of andersom. Een twee-quantum pulsreeks is geoptimaliseerd om een goede selectiviteit en een hoge signaalintensiteit te krijgen. De verkregen signaalintensiteit is echter zo laag dat de methode voor in-vivo toepassingen bijna niet acceptabel is. Een alternatieve methode, gebaseerd op spin-echo metingen is beschreven. Deze methode levert grotere signaalintensiteiten maar is gevoelig voor verstoringen die kunnen ontstaan door bewegingen of door relaxatie effecten (hoofdstuk 4).

In samenwerking met het Academisch Medisch Centrum van Amsterdam is een in-vivo onderzoek uitgevoerd waarin een aantal van de ontwikkelde technieken toegepast worden.

O'Conner heeft een beschermend effect van L-carnitine op acute hepatische encephalopathie waargenomen bij muizen. Met behulp van MRS
en een aantal klinische parameters is dit effect onderzocht bij ratten. Een spin-echo meettechniek met twee dimensionale localisatie is gebruikt om gewone in-vivo spectra te meten. Daarnaast zijn nul- en twee-quantum technieken gebruikt om de verandering in de lactaat concentratie te bepalen. Hoewel een gering beschermend effect van L-carnitine gevonden werd in de EEG-parameters, is het verbazingwekkende effect zoals dat door O'Conner beschreven is, niet waargenomen. De nul en twee-quantum technieken voldeden in deze praktijksituatie goed (hoofdstuk 5).

3. DATA VERWERKINGS TECHNIEKEN.

Een flexibele niet-lineaire kleinste kwadraten methode waarmee in het tijddomein MRS signalen gequantificeerd kunnen worden, is uitgebreid zodat gebruik gemaakt kan worden van voorkennis. Met deze methode kunnen zowel FID-signalen als echo-achtige signalen geanalyseerd worden. De modelfunctie is een exponentieel gedempte sinus. De berekeningen zijn analytisch uitgewerkt om de rekentijd te reduceren. Aan de hand van metingen aan modeloplossingen van medisch interessante stoffen is voorkennis verkregen. Door deze voorkennis te gebruiken wordt een betere nauwkeurigheid verkregen. Een signaal dat uit een aantal sterk overlappende componenten bestaat kon goed gequantificeerd worden. Met een aantal kleine aanpassingen kon dezelfde voorkennis gebruikt worden voor het analyseren van een serie in-vivo metingen (hoofdstuk 6).
Concise list of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>aspartate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CHO</td>
<td>choline</td>
</tr>
<tr>
<td>DQC</td>
<td>double quantum coherence</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalogram</td>
</tr>
<tr>
<td>FID</td>
<td>free induction decay</td>
</tr>
<tr>
<td>GLN</td>
<td>glutamine</td>
</tr>
<tr>
<td>GLU</td>
<td>glutamate</td>
</tr>
<tr>
<td>HE</td>
<td>hepatic encephalopathy</td>
</tr>
<tr>
<td>MQC</td>
<td>multiple quantum coherence</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl aspartate</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMRS</td>
<td>nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>PCR</td>
<td>(phospho) creatine</td>
</tr>
<tr>
<td>PPM</td>
<td>parts per million</td>
</tr>
<tr>
<td>ZQC</td>
<td>zero quantum coherence</td>
</tr>
</tbody>
</table>
Dankwoord

Aan de totstandkoming van dit proefschrift hebben velen, direct of indirect bijgedragen. Al die personen wil ik hartelijk bedanken. Een aantal van hen wil ik bij naam noemen.

Op de eerste plaats natuurlijk mijn promotor, prof. dr. ir. A.F. Mehlkopf. Heel in het bijzonder wil ik Wim Bovée bedanken die voor de dagelijkse ondersteuning gezorgd heeft en van wie ik in de afgelopen jaren het meest geleerd heb. Hoewel Albert de Graaf al een tijdje bij de vakgroep weg is, wil ik ook hem bedanken, van hem heb ik zogezegd de kneepjes van het vak geleerd. Dik van Ormondt wil ik bedanken voor de ondersteuning bij het werk aan de dataverwerkingstechnieken. Rob Chamuleau en Diederik Bosman hebben de in-vivo metingen mogelijk gemaakt. Speciaal Diederik wil ik bedanken voor de vele ritten die hij, vaak 's morgens in alle vroege, richting Delft maakte.

Tot slot bedank ik Joost Alferink die de voorkant van dit boekje ontworpen heeft.
Levensloop