

NMR implantable probe: limit of metabolites detection

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Abstract— In this study a new concept of micro coil is presented in order to measure the small volumes and small concentrations samples by NMR spectroscopy. The goal of our work is to determine the concentration sensitivity and the limit of detection of a planar microcoil of ellipsoidal geometry 1000 x 500mm, fabricated using an electroplating technique and used as NMR receiver coil at 200 MHz. The maximum signal intensity on the NMR images and simulation of RF field distribution enables to define an active volume of 0.09 μL . The localised spectroscopy based on a PRESS sequence shows that the concentration sensitivity is closed to $S_C=0.0272 \text{ mM}^{-1}$, the limit of detection to 11 mM and the normalized limit of detection close to $390 (\text{mM}\cdot\text{s})^{-1/2}$. This micro-system offers the possibility of new investigation techniques based on micro coils' implantation used for in vivo study of local cerebral metabolites occupying small volumes.

Index Terms—implantable micro coils, NMR micro spectroscopy, cerebral metabolism, limit of detection.

I. INTRODUCTION

Nuclear Magnetic Resonance Spectroscopy is one of the most often used techniques to study the metabolism changes in different biological and chemical samples. NMR spectroscopy fulfils an important role through its ability to produce structural information and also to provide data on intermolecular dynamics. Actual studies are concentrated on the analysis of limited sample volumes (orders of μL) – tissues, cell cultures, protein structures [1]. The field of NMR has developed a large array of experimental capabilities but NMR sensitivity still lags significantly behind most other analytical methods by a factor of 100-1000 especially for many important mass-limited and concentration-limited situations. Mass limited samples common to NMR analysis often fall into these categories: natural products, trace contaminants, combinatorial compounds, metabolites which are our subject of interest. According to Hout and Richards, the reduction of the diameter and length of the coil increases the sensitivity [2]. On the other hand, for a fixed coil size and line width the concentration and mass sensitivity is directly proportional to the fraction of volume within the coil is occupied by the sample. This parameter, called the filling factor, depends on the inner diameter of the sample container and on the NMR coil diameter. Adapting the coils dimensions to the samples dimensions, the filling factor will be increased as well as the signal-to-noise ratio and the coil sensitivity (B_1/i) [2], where B_1 is the RF field and i is the unit current.

These considerations establish both the mass-sensitivity (S_m) and the concentration-sensitivity (S_c) as useful figures of merit in NMR probe evaluation [1, 3].

A. The MR microcoil development

Conventional NMR instruments used for small volume analysis give a low signal to noise ratio (SNR) because of their large dimensions compared to the sample size. The amplitude of the NMR signal is maximized when the size of the detection RF coil matches the sample's size. Therefore a micro-NMR device designed for micro-samples is required to improve SNR and consequently to increase the sensitivity. So it is a real challenging task to develop a micro system for NMR spectrum extraction for smaller and smaller sample volume. The most part of the RF micro coils used in high resolution NMR are: the saddle coils, solenoid coils and planar coils.

Webb *et al* have investigated the design and the performances of the solenoid-type micro coils used in NMR spectroscopy [1] as well as K.R. Minard *et al* [4, 5], Olson *et al* used solenoid coils wound directly onto a capillary functioning as both as sample container and coil form [6, 7]. Massin *et al* describe the performances of 500 μm and 1mm inner diameter planar micro coils build using processes based on photolithography techniques [8-10]. Ergolu *et al* have investigated a method for monitoring activity of pancreatic β -cells using a 2mm diameter planar coil at 500 MHz [11, 12]. Grant *et al* made biological investigation in order to describe the planar micro coils performances on single neuron MRI experiments [13-16].

In this article, we describe the fabrication, the B_1 field distribution and the limit of detection (LOD) of a planar microcoil used as receiver coil, fabricated using an electroplating technique on thick photo-resist process. We describe the methodology with the setup developed in order to obtain the sensitivity map of the planar microcoil first by simulation and secondly by MR imaging. The correlation between the simulation and the MR experiments results made possible the estimation of the active volume of the microcoil. The knowledge of the active volume will facilitate the voxel location in MRS data processing. The in vitro ^1H spectrum of six selected proton cerebral metabolites and its quantification will be described in the fourth part of this paper. The values of the concentration limits of detection are promising for *ex vivo*

and *in vivo* study of local cerebral metabolites occupying small volumes.

B. MR figures of merit

NMR experiments including the spectroscopic investigation of mass-limited samples several figures of merit are important to determine the feasibility of a particular experiment using a particular microcoil. For example the structural characterisation of micrometers order samples demands long acquisition times, on the other hand the detection technique and also the biological application implies a restriction in time and thus the information content of the spectra. In order to obtain an appropriate characterization of spectra information figures of merit for NMR mass sensitivity, concentration sensitivity, and limits of detection are presented and evaluated. It is well known from NMR literature that the signal strength and detection depends of the several factors as: the field uniformity in the excitation process, the sensitivity, size and geometry of the RF receiver coil, the sample concentration and volume, the number of magnetically equivalent nuclei which give rise to a particular resonance, the line width of the resonance. At this point, it is necessary to determinate the sample observation efficiency, meaning the ratio between the total sample volume and the volume of sample observed by the RF coil. Because of magnetic susceptibility discontinuities at air and container interfaces, the sample usually extends beyond the coil region to provide quality data; a limit of detection (LOD) in terms of sample mass and concentration which yields an S/N of 3, for the entire sampling system can be significant and has to be determined.

In a review on small samples NMR detection Lacey *et al.* [3] had defined two different performance indicators: mass sensitivity (S_m) and concentration sensitivity (S_c):

$$S_c = \frac{SNR}{C} \quad (1)$$

$$S_m = \frac{SNR}{mol} \quad (2)$$

The limit of detection (LOD) representing the minimum concentration and sample mass necessary to yield a SNR of 3 is defined as:

$$LOD_c = \frac{3}{S_c} \quad (3)$$

$$LOD_s = \frac{3}{S_s} \quad (4)$$

The analysis of mass-limited samples requires the accumulation of a number of acquisitions or the concentration must be increased to give a meaningful result. Consequently, these performance parameters can be more explicitly defined as time-normalized concentration sensitivity and mass sensitivity,

$$nS_c = \frac{S/N}{C \cdot t_{acq}^{1/2}} \quad (5)$$

$$nS_m = \frac{S/N}{mol \cdot t_{acq}^{1/2}} \quad (6)$$

Where C is the sample concentration, the mole amount refers to the portion of the sample which resides within the volume observed by the coil and $t_{acq}^{1/2}$ the total experiment time.

This information is essential in experiments including animal models, like the metabolism variation, experiments which require a specific temporal resolution.

II. MATERIALS

Micro-coil fabrication:

The presented micro coil is a planar coil with ellipsoidal geometry $1000 \times 500\mu m^2$, (Figure 1) made using an electroplating technique [17], with four concentric turns, (traces width of $22\mu m$, trace thickness $46\mu m$ and spacing between turns of $20\mu m$).

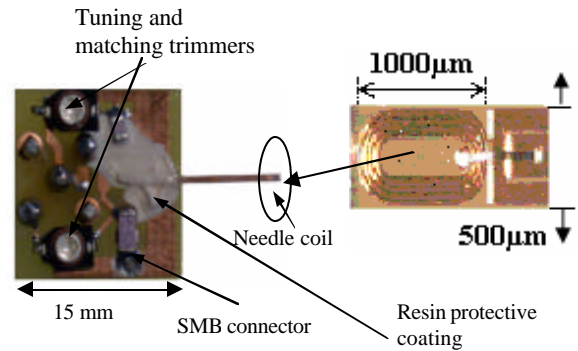


Figure 1: (a) Micro coil with tuning/matching circuit (b) SEM view of the needle coil

The electric characterisation of the micro coil was performed using a HP 4195A network and have the following values: $L=7,5nH$, $R=0,62\Omega$, $Q=24$.

For a micro coil used as a single coil transmits-receive mode the field of view obtained is a relatively small part of the active coil volume because of the non-uniformity of the excitation. With separate transmitter (large) and receiver (small) coils the sensitivity and RF field uniformity can be improved. In our experiments the planar micro coil is used as a receiver, the excitation is realised using a Rapid Biomed bird cage coil (inner diameter 6.9cm). When the planar micro coil is used for detection only and another coil for excitation there is a strong electromagnetic coupling caused by mutual inductance since both coils operate at the same resonance frequency. We can eliminate the mutual induction by detuning the micro coil during the excitation phase and quenching the emission coil during the detection phase [18]. This problem is easily solved using a PIN diode as we can see in figure 2.

The circuit in figure 2 is the micro coil resonant circuit model, where C_{tune} and C_{match} are the tuning and matching variable capacitors (5 to 10 pF). The third capacitor, $C=39$ pF, is imposed by the presence of the PIN decoupling diode (Pin diode Philips semiconductors BAP 64-03) in order to avoid the short circuit of the command tension ($V_{decoupling}$) by the

microcoil. In the decoupling mode, the PIN diode is in conduction, commanded with a tension of +3.8V. The tuning capacity will be shortcut by the PIN diode, the frequency of resonance of the micro coil becomes:

$$f_{res} = 1 / (2\pi \sqrt{L_{coil} \cdot C}) \quad (6)$$

The decoupling mode is activated when the NMR sequence generates the RF pulses in order to attenuate the induced currents in the receiver micro coil.

In the reception mode the PIN diode is commanded with a command tension ($V_{decoupling}$) of -30V.

The resonance frequency becomes:

$$f_{res} = 1 / (2\pi \sqrt{L_{coil} \cdot C_{eq}}) \quad (7)$$

and it is equal to 200.3MHz, the proton resonance at 4.7T, where:

$$C_{eq} = (C \cdot C_{tune}) / (C + C_{tune}) \quad (8)$$

The presence of the filtering inductance $L_{filtering}$ is used to avoid the possible losses of the NMR signal on the command way and also to block the noise induced by the command way.

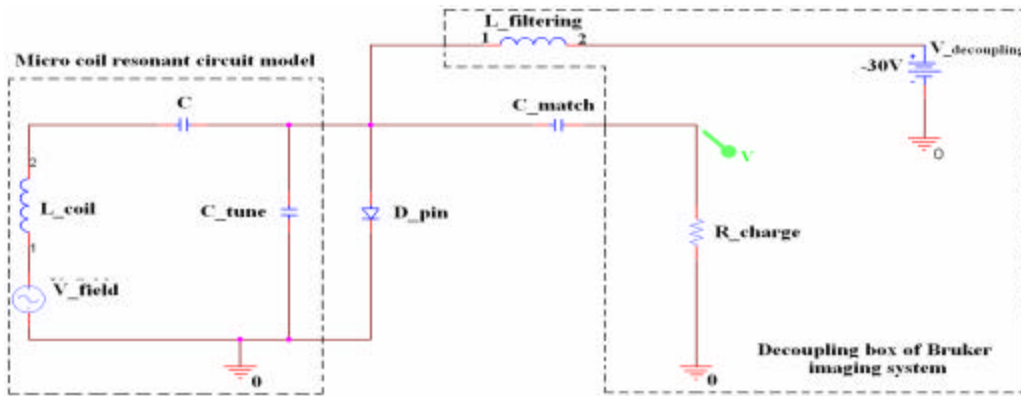


Figure 2: Receiver NMR micro coil electrical model and its decoupling circuit.
 (left) Electrical model of planar microcoil during the decoupling mode, Pin diode in conduction.
 (right) Electrical model of microcoil during the receiving mode: Pin diode blocked.

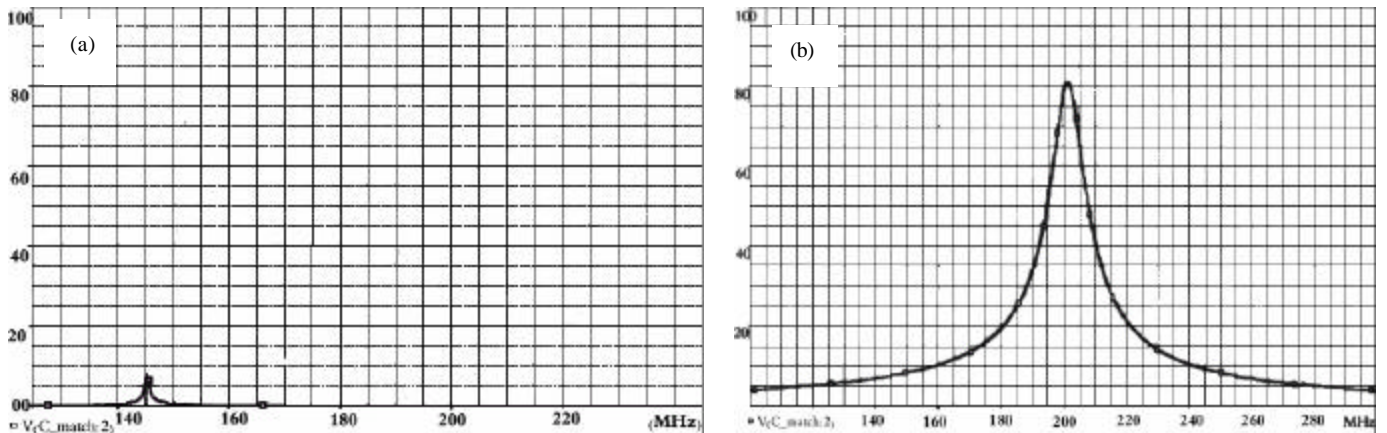
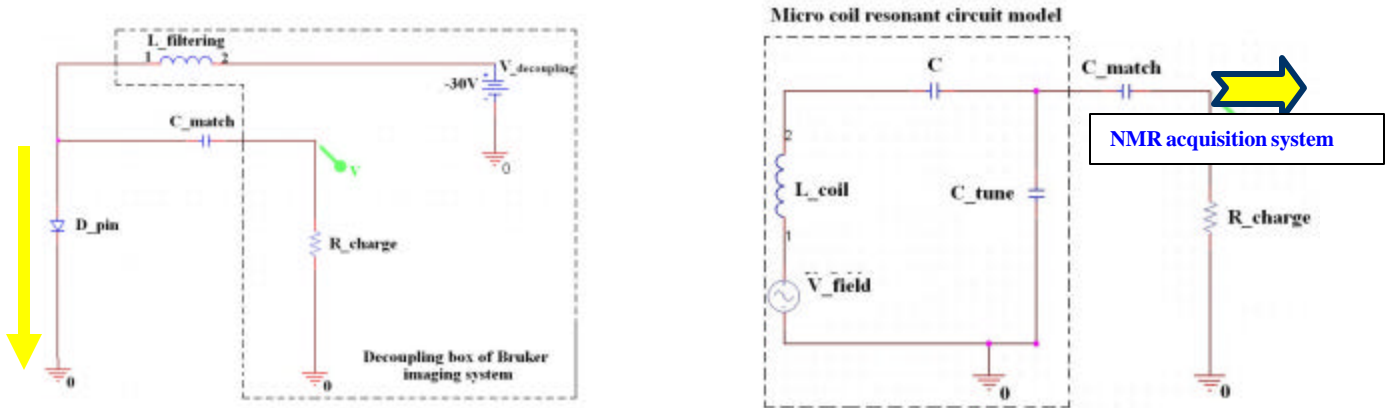


Figure3: S11 plots showing the effects of decoupling circuit on the microcoil resonance frequency
 (a) Pin diode in conduction, $f = 145$ MHz, microcoil is off, (b) Pin diode in conduction, $f = 200$ MHz, Microcoil is in receiving mode.

III. METHODS

The knowledge of the RF coil field cartography in terms of uniformity and amplitude and the knowledge of the corresponding sensitivity distribution make possible a precise voxel location for MR imaging and spectroscopy investigations.

RF field simulation

The RF field map of the presented micro coil was computed with the Matlab software using the concentric loop model [8] showed in figure 4 using the Biot-Savart Law.

The amplitude of the field distribution strictly depends on the geometrical parameters of the coil (width, trace height and trace spacing) and each conductive part contributes to the field generation. So, all conductive parts of the micro-coil may be decomposed in elementary segments carrying currents. The principle of superposition permit to add together the corresponding elementary magnetic vectors and thus the global magnetic field of the micro-coil can be calculated using the Biot-Savart law. The sensitivity for the modulus of the RF map is obtained by projecting B_1 onto the plane perpendicular to the main magnetic field B_0 and numerically generating the variation of the magnetic field in the other two axes (figure 5). In NMR experiments only the perpendicular component of the magnetic field gives the NMR signal. In order to correlate the MRI images with the Matlab 2D and 3D simulations it is necessary to numerically generate the distribution of the magnetic field in a plane perpendicular to the coil surface. The distance from the micro coil surface is $d = 1.25\text{mm}$ in figure 5.

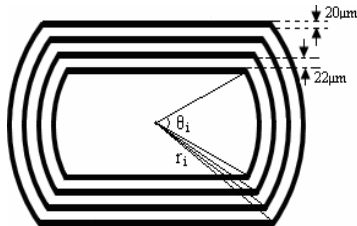


Figure 4: The concentric loop model used for the Matlab simulations. Each conductive part was approximated with two segments and two arc circle.

Figure 6 shows the 3D spatial field distribution obtained for a distance of $d_1 = 500\mu\text{m}$ (a) and $d_2 = 700\mu\text{m}$. We can notice the variation of the field amplitude as a function of the distance.

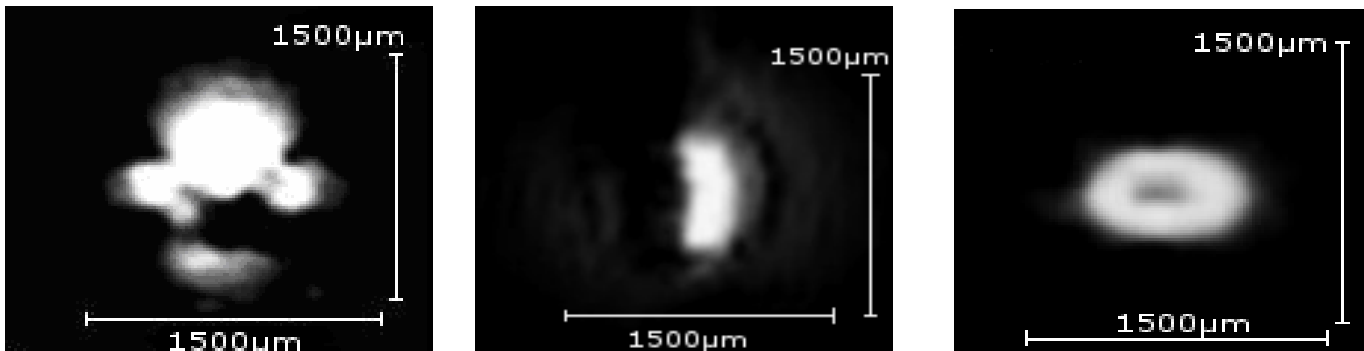


Figure 7: MRI images obtained at 4.7T: coronal plan (a), sagittal plan (b) and transversal plan (c).

This is in good agreement with the sensitivity map of a conventional surface coil.

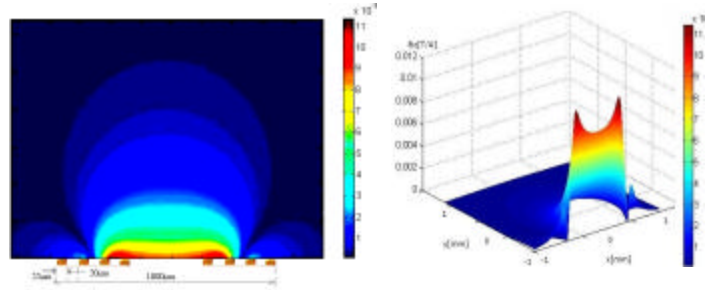


Figure 5: 2D sensitivity map of the B_z component from a plane perpendicular to the microcoil axis (left) and from a plane parallel to the microcoil axis (right).

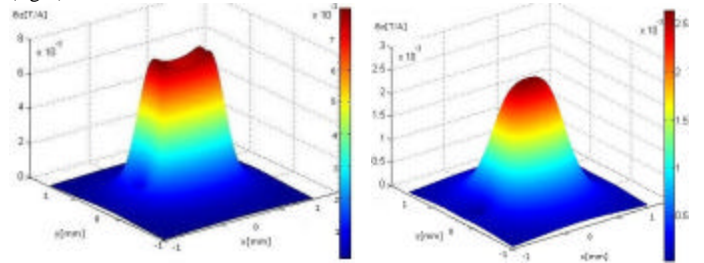


Figure 6: 3D spatial field distribution obtained for a distance of $d_1 = 500\mu\text{m}$ (left) and $d_2 = 700\mu\text{m}$ (right) parallel to the microcoil axis.

RF field map by NMR imaging

NMR experiments were performed using a BRUKER 4.7T Biospec System with a 200 mT/m gradient set. The planar micro coil is used for signal detection only. It was immersed in a water sample (Figure 8) and NMR images showing the signal distribution were acquired using a FLASH sequence with: FOV = 1.19cm, TR/TE = 100/6ms, 30° flip angle, 1mm thick slice, coronal plane, 100 μm spatial resolution, 2 averages, acquisition time 2 minutes 56 seconds.

The active volume of the planar microcoil is determined measuring the volume for which the MR signal amplitude is maxim. The sensitivity map in figure 7 allows estimating the signal distribution in all the three dimensions: coronal, transversal and sagittal and consequently the voxel dimension. The MRI results will be correlated with the simulations showed above.

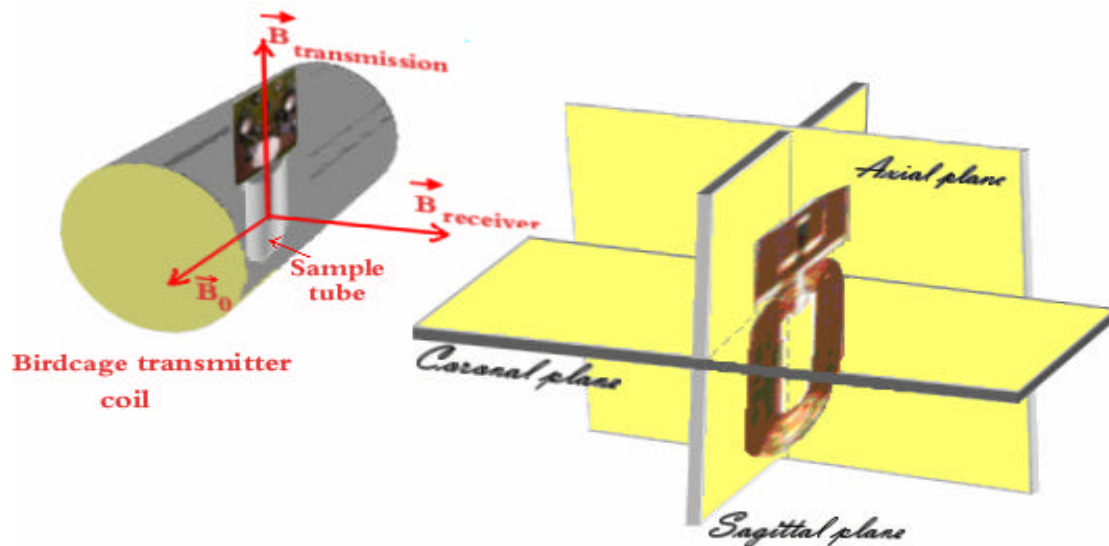


Figure 8: Setup used for the MR experiments including the slice selection used to determine the microcoil sensitivity map by MR imaging

IV. RESULTS

The MRI results show that the maximal signal intensity covers a volume having these dimensions: $650\mu\text{m} \times 350\mu\text{m} \times 400\mu\text{m}$ ($h \times l \times t$, where h is the height from the coronal plane, l the length in the transversal plane and t the thickness in the sagittal plane – figure 7(a, b, c)). The measured dimensions make possible to estimate a voxel size of 0.09 mm^3 and an active volume of the micro coil of $0.09\mu\text{L}$. This is a volume underestimation; the voxel was approximated to a parallelepiped form which is not the real radiation form. A more precise calculus should include the real dimension for each lobe which characterizes the microcoil sensitivity map. The signal-to-noise ratio (SNR) was measured on the MR images by selecting a region of interest (ROI) in the coil centre to measure the mean signal and a region outside the sample extend for the root mean square noise measurement. In that case $\text{SNR} = 80$.

¹H MR Spectroscopy applications

The first step in the *in vitro* spectroscopic validation of the microcoil is to determine the concentration sensitivity, the limit of detection and the normalized limit of detection.

The phantom solution used for this purpose contains a mixture of six MR - observable ¹H metabolites in human brain: lactate (Lac), N-acetyl aspartat (NAA), gamma amino-butyric acid (GABA), creatine (Cr), choline (Cho) and taurine (Tau) (Sigma Aldrich) [19]. Trimethylsilyl-propanesulfonic acid sodium salt (DSS) and sodium formate were added as chemical shift references.

The solution was prepared with a concentration of 100mM in water except for creatine and taurine for which the concentration was 50mM in water ($\text{pH}=7.0\pm 0.1$, 0.5ml). All the *in vitro* metabolite signals should be measured using identical acquisition parameters as the *in vivo* case, at the same pH and temperature conditions as the ones in the rat brain. To further evaluate the figures of merit described before and the

performances of the microcoil the ¹H spectrum in figure 9 was obtained.

The quantification of the ¹H spectrum raises some difficulties; the major problems encountered being the strong overlapping peaks. The signals with low signal to noise ratio and the large amplitude of the water peak needs to be removed. The water signal was suppressed by variable power RF pulses with optimized relaxation decays (VAPOR). Outer volume suppression (OVS) combined with a short echo-time PRESS sequence was used for localisation. Removal of residual water components was performed in a pre-processing step using the Hanke-Lanczos Singular Value Decomposition algorithm – HLSVD. The sequence parameters are: $\text{TR/TE} = 5000/20\text{ms}$, voxel size = 1mm, 256 averages, acquisition time 21minutes. Eddy current compensation and static magnetic field drift correction were applied during the acquisition.

The MRS data processing is performed using prior knowledge of the metabolite signals to be analyse. The QUEST method exploits the quantification method in the time domain and a basis set of cerebral metabolites signals. The six metabolites used in the phantom solution (Lac, NAA, GABA, Cr, Cho, Tau) are used as prior knowledge in the algorithm and they were obtained using a simulated basis set theoretically measured by quantum mechanics. The method QUEST models the MRS signals by a linear combination of metabolites signals measured by NMR-SCOPE using the adjustment factors [20]. NMR-SCOPE based on product-operator formalism can handle various MR pulse sequences like the PRESS sequence used here.

In table 1 all figures of merit are calculated and presented for each metabolite signal including the concentration sensitivity, mass sensitivity, concentration limit of detection, mass limit of detection, normalized concentration limit of detection and normalized mass limit of detection. The performed quantification gives the value of 27 for the signal-to-noise ratio (SNR) of the Choline signal; the concentration limit of detection is currently at 11 mM. The normalized concentration limit of detection (nLODc) has a value of $390 \text{ (mM*s)}^{-1/2}$.

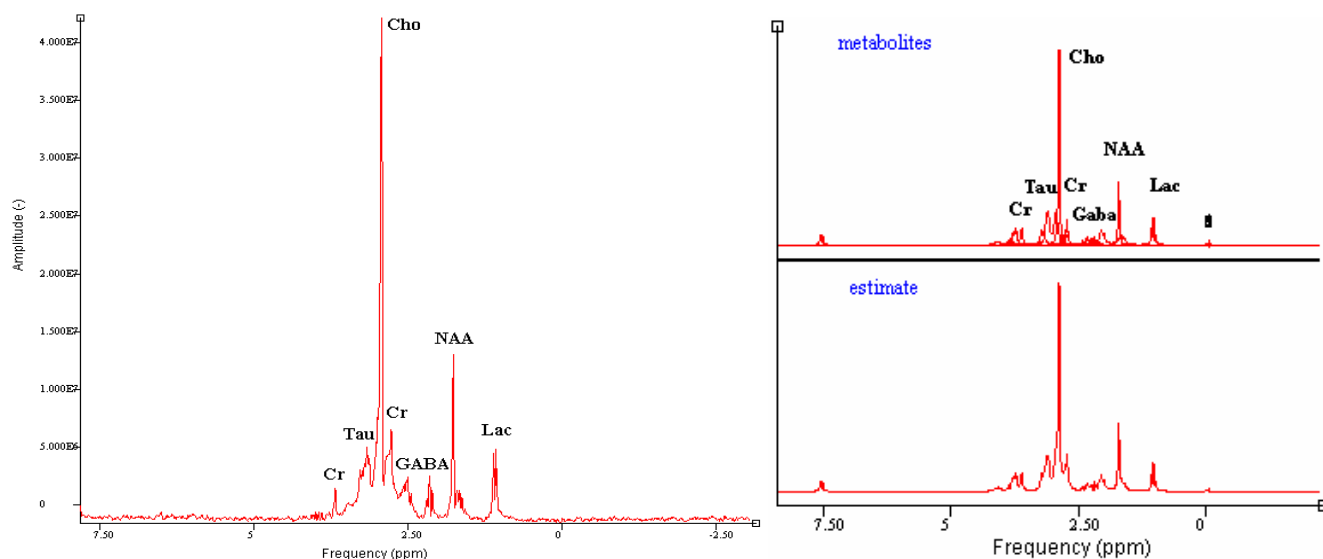


Figure 9: ^1H spectrum of six cerebral metabolites: Lactate, NAA, GABA, creatine, choline and taurine. Left: the acquired spectrum; right: Quest quantification.

<i>Metabolites</i>	<i>Creatine</i>	<i>Choline</i>	<i>NAA</i>	<i>Taurine</i>	<i>Lactate</i>	<i>GABA</i>
<i>Sc</i> [mM^{-1}]	0.511	0.272	0.061	0.06	0.037	0.015
<i>Sm</i> [nmol^{-1}]	2.858	1.49	0.33	0.322	0.2	0.083
<i>LODc</i> [mM]	5.86	11	49	50	79	200
<i>LODm</i> [nmol]	1.05	2.01	9.09	9.3	15	36.14
<i>nLODc</i> [$(\text{mM} \cdot \text{s})^{-1/2}$]	207	390	1730	1770	2800	7000
<i>nLODm</i> [$(\text{nmol} \cdot \text{s})^{-1/2}$]	37.2	71.33	322	330	532.35	1277

Table 1: The values of the sensitivity and the limit of detection for each spectrum component identified with the QUEST method.

V. CONCLUSION

The presented ^1H spectrum of the solution containing MR-observable cerebral metabolites proves the actual performances of the microcoils. A simulation model based on Biot-Savart law has been developed in Matlab and the sensitivity map has been correlated well with the two dimensional profiles of the receiver coil in three selected plans acquired with a FLASH sequence. The active volume was estimated taking into account the signal distribution in all the three directions. The MRI results are in good agreement with the simulation; the active volume found of $0.09\mu\text{L}$ makes possible a precise voxel selection for the localised spectroscopic experiments. At this scale, knowledge of the maximal signal distribution is essential for all NMR investigations. The values of sensitivity concentration, $S_c=0.272$, the concentration limit of detection of 11mM and the normalized limit of detection of $390 (\text{mM} \cdot \text{s})^{-1/2}$ for the Choline case are promising for *in vivo* study of local metabolites in small volumes of living tissues. These first results validate the methodological aspects for the use of receiver implantable microcoils in high resolution MR spectroscopy. Although the limit of detection in the *in vivo* cases is between 2 and 10 mM [19], the setup proposed here is a proof of concept for the future experiments, more precisely

for the microcoil implantation in the rat brain. The purpose of the microcoil implant is to detect the variation of proton cerebral metabolites by MR spectroscopy.

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