In vitro metabolites limit of detection by localised NMR spectroscopy using micro coils

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Introduction

Considering the need to explore small quantities of biologic samples by MR spectroscopy, it is possible to create probes with a working volume compatible with such limitations. The investigation of mass-limited and concentration-limited samples by MRS requires several performance parameters in order to validate the feasibility of using MR micro coils. These criteria are: concentration sensitivity S_c, $S_c=SNR/C$, (C - sample concentration), mass sensitivity S_m, $S_m=SNR/mol$ (*moles* - amount of mass or moles of the sample), concentration limit of detection LOD_c, $LOD_c=3/S_c$, the mass limit of detection LOD_m, $LOD_c=3/S_m$ and their time normalised values $nLOD_c$ and $nLOD_m$ [1]. In this study, we present the performances of a new concept of micro coil offering the possibility to investigate volumes of microliter order by highly resolved MR spectroscopy.

Materials and methods

The micro coil is a planar one of ellipsoidal geometry (1000x500 µm²) made using an electroplating technique, with four concentric turns [2] (Figure 1, *a, b, c*). MR experiments were performed using a BRUKER 4.7 T Biospec System with 270 mT/m gradient set. The planar micro coil was used for signal detection only. RF transmission was performed by a Rapid Biomedical birdcage coil (∅inner= 8.4 cm); the two coils having an active decoupling [3]. The micro coil was immersed in a water sample and MR images showing the signal distribution (Figure 1, *c*) was acquired using a MSME sequence: FOV = 1.19 cm, TR/TE = 1000/14 ms, in plane isotropic digital resolution 172 µm/pixel, 2 averages, slice thickness 0.3 mm, 8 slices.

Localised spectroscopy acquisitions (Figure 2) are performed with a short echo-time PRESS sequence: TR/TE=7 s/20 ms, voxel 8 µl, 256 averages for 30 min of scan time. The proton water peak was suppressed using a VAPOR sequence [4]. The spectrum was quantified using the jMRUI software and the AMARES method [5].

Results

The active volume of the planar micro coil was estimated from the MR signal intensity value in Figure1*c*. The MR signal intensity (70 % of the maximum observed intensity) occurs in an active volume V_{active}= 2.07±0.06 µl. *In vitro* spectrum of a solution containing a mixture of eleven MR - observable ^{1}H metabolites in human brain (lactate (Lac), N-acetyl aspartate (NAA), gamma amino-butyric acid (GABA), choline (Cho), creatine (Cr) Glutamine (Gln), Glutamate (Glu), myo-Inositol (m-Ins), Aspartate (Asp), Glucose (Glc) and taurine (Tau) with 50 mM in water, pH =7.0±0.1) is displayed in Figure 2.

Estimated limits of detection for a 30 minute experiment [1] and time normalized limit of detection are given in Table 1 for Choline and NAA, which may be used as reference signals *in vivo* [6], LW is the half height linewidth.

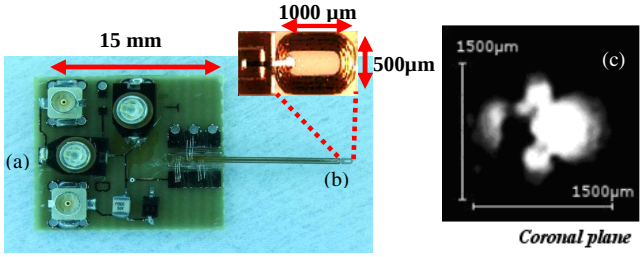

Figure 1: (a) Micro coil with its tuning/matching circuit (b) SEM view of needle coils (c) micro coil sensitivity distribution in the coronal plane.

Metabolite	S_C (μM^{-1})	LOD_c (mM)	nLODc ($mM \cdot s^{-1/2}$)	LW (Hz)
Choline	57±0.5	3.3±0.3	138,3±10	5
NAA	289±23.3	10.4±0.85	442,5±42	3.8

Table 1: Concentration sensitivity, limit of detection, time normalised LOD_c and line width values for Choline and NAA.

Note the particularly narrow line widths for Cho and NAA signals compared to other reported spectra acquired using micro coils [7].

Discussion

These results validate the use of micro coils for *in vitro* studies and they are promising for *in vivo* applications. The actual LOD_c value of Choline is close to the concentrations found in the rat brain [8]. The Choline concentration *in vivo* is close to 2 mM and the NAA concentration to 7.5 mM. A method to reach this value is to improve TR in the PRESS sequence. The signal analytical expression was calculated for a flip angle θ and the TR value was further estimated based on the *in vivo* metabolite T_1 values. It is known that the maximal signal occurs for an intermediate Ernst angle θ_E [9], here given as:

$$\cos\theta_E = e^{-\frac{TR-\tau}{T_1}} \quad \text{, where } \tau=T_E/2 \qquad (1)$$

The estimated scan times to detect the Choline and NAA at *in vivo* concentration are summarized in Table 2.

Metabolite	T_1 (s) (rat *in vivo*)	TR (s)	θ (°)	Scan time (min)
Choline	1.91	1.27	59	44
NAA	1.33	1.2	66	24

Table 2: T_1, TR and θ optimum value to be used in a PRESS sequence and the estimated scan time necessary to detect Choline and NAA at *in vivo* concentration.

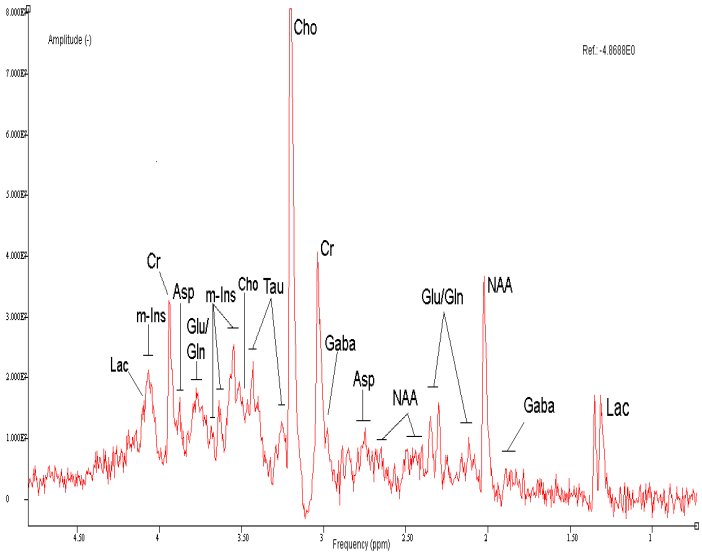

Figure 2: Acquired ^{1}H spectrum of eleven cerebral metabolites: Choline, NAA, Creatine, Lactate, GABA, Glutamine, Glutamate, Taurine, myo-Inositol, Aspartate, Glucose, c= 50 mM, pH =7.0±0.1. Note the truncation of the Choline peak. The metabolite assignments are identified using abbreviations used in the text.

For in vivo analysis, Choline and NAA may be present in the same sample, and then an intermediate flip angle value has to be used.

Conclusion

In this work the active volume of the designed microcoils has been estimated by MRI and found about 2 microliters. For spectroscopy, the single voxel approach using independent transmitter coil was efficient. It has been employed to evaluate the in vitro limits of detection on a series of six microcoils showing a very suitable reproducibility from one coil to another and opening the possibility to work at in vivo concentration.

References

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