The Macromolecular ^{1}H NMR lineshape in cartilage

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Introduction:
The analysis of the magnetization transfer contrast (MTC) under incomplete saturation of the macromolecules (as is common in clinical setups) requires the knowledge of the macromolecular spectral lineshape. In previous studies this information was retrieved by fitting the MTC data to models where the lineshape characteristic was kept as a free parameter. In the current study we present a method and subsequently results that allow measurement of the macromolecular lineshape.

Theoretical background and pulse sequences:
In order to measure the macromolecular lineshape it is necessary to suppress the water and metabolites peaks that mask the macromolecular spectra. As was previously shown this goal can be achieved by the DQF-MT pulse sequence (2) that combines double quantum filtering (DQF) with zero quantum filtering (ZQF) i.e. 90-τ-90-t_{DQ}-90-τ-90-t_{LM}-90-Acq (Eq. 1). The first two pulses and the interval (τ) between them are used to excite double quantum coherence (DQC) while the two following ones and the interval between them (τ) reconvert it to longitudinal magnetization. In previous studies the observed magnetization was shown to depend on the dipolar interaction (ω_D) among the protons in the various types of molecules presented in the tissue as $Sin^2(\omega_D\tau)$ and thus by selecting a suitable value of τ one can distinguish between water molecules and macromolecules whose ω_D differs by two orders of magnitude. However, since the macromolecular spectrum is inhomogenously broadened by the dipolar interaction, only spins for which $\omega_D^{mm}\tau\sim1$ will be excited while those with $\omega_D^{mm}\tau<<1$ will be suppressed. Thus since the range of ω_D is 0-40 kHz, setting τ to be a few μs only segments with values of ω_D at the higher end of its range are excited and the spectrum looks as if there is a splitting (see Fig. 1 for articular cartilage). However, spin diffusion (SD), caused by the dipolar interaction between the protons, leads to the establishment of spin temperature, where the observed spectrum is identical to its thermal equilibrium shape. The SD process occurs during the interval t_{LM} between the last two pulses. The effect of saturation was examined by preceding the pulse sequence shown in Eq. 1 by a saturating pulse whose carrier frequency was varied over the whole macromolecular spectrum. The level of attenuation of the macromolecular spectrum was plotted against this frequency obtaining a spectrum that is the analogue of the conventional z spectrum. Thus we denote this spectrum as z-mm spectrum.

Experimental:
The experiments were conducted on a piece of bovine articular cartilage excised from a femoral head obtained from a slaughter house and used either in its native form or after immersing it in deuterated saline solution for several hours. The experiments were conducted on a Bruker Avance 360WB spectrometer operating at 8.4T. Pulses of 14μs duration were used in the pulse sequence shown in Eq. 1.

Results and Discussion:
Since we didn't find significant changes in the macromolecular ^{1}H spectral lineshape upon replacing H_2O by D_2O, and since the latter samples are free of the effect of fast exchanging protons, in the following we shall present results obtained for the latter type of samples.
In Fig. 2 we show the macromolecule spectrum obtained after spin diffusion allowed establishing spin temperature. As can be seen from figure 2 (see caption) the lineshape is well described as Gaussian with width at half height of 27.3kHz enabling to reduce the number of free parameters in the analysis of conventional MTC. It is noticeable that the macromolecular peak is very close to that of the water implying that maximum attenuation obtained in conventional MTC experiments is not only a result of direct effect of saturating the water but also has contribution from saturation of the macromolecules. In Fig. 3 we show the **Z-**mm spectrum of the cartilage. A fit to Gaussian gave width of 26kHz and is almost identical with the linewidth of the macromolecular spectrum shown in Fig. 2. This result reflects the fact that spin temperature is valid for the macromolecule. I.e., regardless of the offset at which the saturation pulse is applied spin diffusion will lead to the same lineshape. However, some differences between the z-mm and the macromolecular spectra are found in range of -4 to +4 ppm as a result of saturating protons that transfer magnetization either to the macromolecules or to the water by SD or NOE.

Conclusions: (1) The lineshape of macromolecules can be measured by the DQF-MT pulse sequence so that its characteristics can be used in the analysis of MTC experiments. (2) Saturation of protons that affect the macromolecules may indirectly affect the magnetization transfer to water.

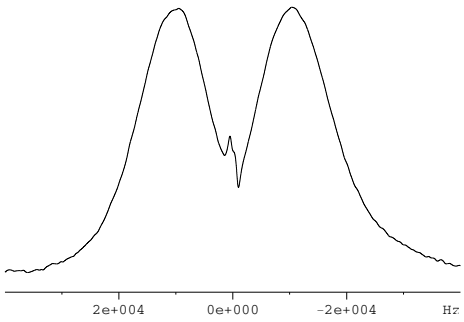

Fig. 1 Lineshape of the macromolecular fraction in cartilage obtained using the pulse sequence given in Eq.1 with the following parameters:τ=5μs, t_{LM}= 3μs, t_{DQ}=2μs, # of averages 64.

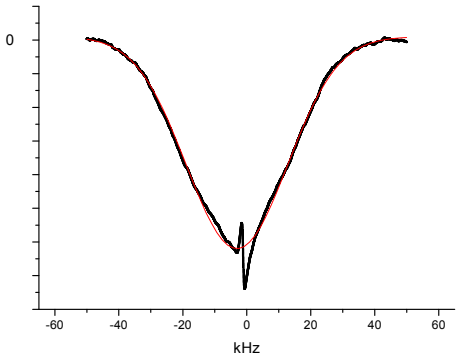

Fig. 2 Lineshape of macromolecule obtained using the sequence in Eq.1 with the following parameters: τ=5μs, t_{LM}=300μs, t_{DQ}=2μs, # of averages 64 (for convenience of comparison with Fig. 3 the spectrum phase was inverted).

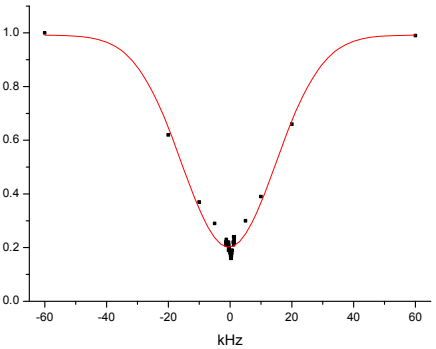

Fig. 3 z-mm spectrum obtained using with r. f. intensity of 55Hz. other parameters are similar to those given in caption to Fig. 2.

References:
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