

# Relaxation time constants T1 and T2\* of bound and free water in cortical bone at 600 MHz and 700 MHz.

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## INTRODUCTION

Cortical bone is a composite material consisting of mineral (~43% by volume), organic matrix (~35%) and water (~22%)<sup>1</sup>. It has multiple water components including water loosely bound to the organic matrix, water tightly bound to mineral, and water residing in the pores of cortical bone. Bound and pore water make different contributions to the biomechanical properties of cortical bone and in understanding bone behavior. It is of critical importance to separate them and quantify their T1 and T2\* relaxation times as well as water content. Accurate measurement of water content also requires measurement of the T1 and T2\* values of bound and pore water. The purpose of this study was to accurately measure the T1s of bound and pore water in cortical bone using NMR spectrometers.

## METHODS

Fresh bovine cortical bone samples (n=3) from a local slaughter house were sectioned into 2.5x2.5x5 mm<sup>3</sup> segments and stored in NMR tubes filled with Fomblin to minimize susceptibility differences and dehydration. A rubber eraser, which has similar MR relaxation times as cortical bone was used as a calibration phantom for the measurement of bone water content. It was cut into similar size and stored in the NMR tubes for analysis. T1 and T2\* of bone and rubber were measured with two high field NMR spectrometers: a Magnex 54mm bore superconducting system operating at 14.1 T (600 MHz) and a 16.5 T (700 MHz) with a Bruker Avance III interface and a Bruker Topspin Version 2.1.6 software platform. An inversion recovery sequence was used to measure T1/T2\* using a 180° (duration=29μs) pulse to invert the longitudinal magnetization followed by a 90° (duration=14.5 μs) for signal excitation. Free induction decays (FIDs) were measured with a series of inversion

times (TI) between 1μs and 20s. NMR spectra were generated for each TI, with bound water (broad spectra) and pore water (narrow spectra ~ 4.75 ppm) readily identifiable. T1s for bound and pore water were calculated respectively by interpolating the TI for zero crossing where  $T1 = T1_{zero} / \log(0.5)$ . A bi-component exponential fit to the FID was performed with the longest TI. The bi-component analysis includes a fifth offset term to account for the non-zero contribution of signal amplitude from noise sources.

## Results and Discussion

FID data of bovine cortical bone shows a bi-component decay, consistent with the existence of two distinct water components (bound and pore water). Collagen protons (T2\* <10 μs) provide little contribution to the FID with a 29 μs inversion pulse and a 14.5 μs excitation pulse.

**Figure 1** shows spectra of cortical bone and rubber phantom at a series of TIs. Bound and pore water peak can be easily identified. The amplitude change versus time was used for subsequent T1 analysis.

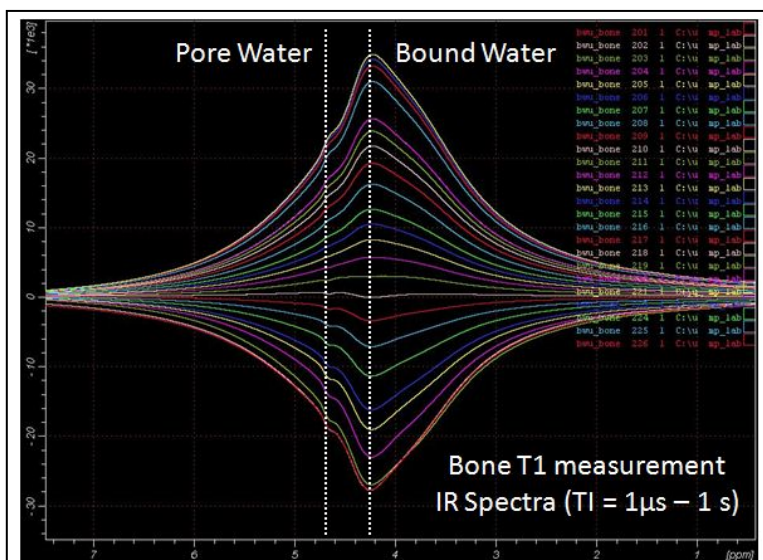
**Figure 2** shows inversion recovery of bound and pore water components. Zero-crossing times, and thus T1s can be accurately measured.

**Table 1** shows the T2\* and T1 values of bound and free water in cortical bone, and short and long T2\* components in rubber. T2\* decreases in cortical bone with increasing operational frequency. T2\* of bound/free water was measured to be  $6.2 \pm 0.1$  μs and  $80 \pm 3$  μs at 600 MHz, and decreased to  $5.4 \pm 0.1$  μs and  $63 \pm 5$  μs at 700 MHz. The T1s of bound/pore water were 628/632 ms at 600 MHz and 661/672 ms at 700 MHz. At both field strengths, the pore water contributes about 10% of the NMR signal. As TI approaches zero, the data deviated from the expected  $A(1-2e^{-TI/T1})$  curve as seen in **Fig 2A**. This deviation is not evident in the rubber phantom (**Fig 2B**) which suggests this is not a systematic error. Future investigation into the possibility and justification for bi-component T1 analysis is ongoing. NMR studies at other field strengths (e.g., 500, 400 and 300 MHz) will also be performed, and bound and pore water T1s as a function of field strength will be assessed.

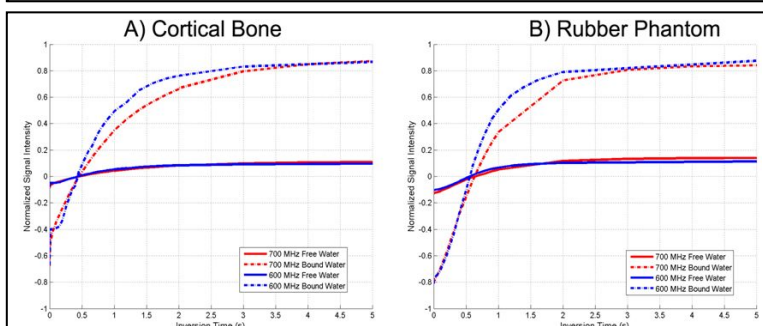
## Conclusion:

Bound and pore water in cortical bone have quite different T2\*s, but similar T1s at 600 and 700 MHz. Rubber eraser has only an 8% higher T2\* and a 28% higher T1, and thus is an appropriate phantom for measurement of bound water content in cortical bone.

**References** : [1] ASBMR Bone Curriculum, 2004. [2] R. Krug et al JMRI, 2011 ; 34(3) :691-695 [3] J. Du et al MRM 2013 : 70 :697-704



**Fig 1** Bone spectra acquired with inversion recovery FIDs with TIs ranging from 1 μs to 1 sec. Bound and pore water peaks (dashed lines) are easily identified. Change of their peak heights vs. TI can be used to calculate the zero-crossing time and thus accurately quantify the T1s of bound and pore water components.



**Fig 2** Inversion recovery of bound and pore water for human cortical bone (A) and rubber phantom (B). Zero crossing was used to determine their T1s. Interestingly the rubber phantom shows two distinct peaks similar to those of bone. Bound and pore water have similar T1s of 628/632 ms at 600 MHz and 661/672 ms at 700 MHz.

A) BONE		
Parameter	600 MHz	700 MHz
T2* Bound Water	6.2(0.1) μs	5.4(0.1) μs
T2* Free Water	80(3) μs	63(5) μs
% Bound Water	90%	89%
T1 Bound Water	628 ms	661 ms
T1 Free Water	632 ms	672 ms
% Free Water	10%	11%
B) RUBBER		
Parameter	600 MHz	700 MHz
T2* Bound Water	6.7(0.1) μs	5.79(0.02) μs
T2* Free Water	84(2) μs	66(1) μs
% Bound	88%	86%
T1 Bound Water	805 ms	915 ms
T1 Free Water	804 ms	923 ms
% Free Water	12%	14%

**Table 1** T1, T2\* and water fractions for bovine bone (A), and T1, T2\* and proton fractions for the rubber phantom (B)