# Clean Separation of Bound vs. Free Sodium by <sup>23</sup>Na Inversion Recovery

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#### Introduction

Monitoring the bound sodium pool can be an important tool for assessing the onset of tissue disorders. Practical clinical <sup>23</sup>Na MRI methods furthermore often do not allow one to use sufficiently small voxel sizes such that only the tissue of interest is seen, but a large signal contamination can arise from sodium in synovial fluid. A clean separation between the signal from bound or ordered <sup>23</sup>Na over that of free <sup>23</sup>Na is particularly important and can greatly enhance the potential of <sup>23</sup>Na-MRI as a diagnostic tool.

In the current study, we demonstrate the feasibility of employing the inversion recovery (IR) sequence to selectively detect the ordered sodium signal or free sodium signal in cartilage tissue. The most likely reasons for this difference are immobilization and larger induced quadrupolar interactions of the bound sodium. In contrast to techniques that rely on the residual quadrupolar coupling [1] it is easy to select the free sodium signal as well with this method.

## Samples

All experiments were carried out on a Bruker Avance 500 MHz spectrometer with a BBO probe tuned to sodium frequency. We optimized the relaxation delay r between these two pulses in order to achieve selective detection of the ordered sodium or the free sodium signal. All pulse durations were calibrated based on the free sodium signal. Hard pulses were used (7.55  $\mu$ s  $\pi$ /2 pulse). For the 1D imaging profiles the gradient strength was 2 G/cm. The free sodium sample was prepared by filling the phosphate-buffered saline solution, (54.8 mM NaCl) into a 5mm NMR tube. The ordered sodium sample was prepared by cutting a sample of diameter of 3mm and height 4 mm from a bovine patellar cartilage, placing it at the coil centre position of the 5mm NMR tube, and adding a fluorinated oil solution (fluorinert FC77, Aldrich) to fill the void spaces. The free-ordered sodium mixture sample was prepared by cutting a sample of the same dimensions as above from a bovine cartilage sample and placing it near to the bottom of a 5mm NMR tube. The fluorinated oil solution was added to a level of 24 mm (top meniscus) and 2.5 mm of a 54.8 mM saline solution was added at the top (measured from the top fluorinert meniscus to bottom of the water meniscus). Fluorinert FC77 is heavier than water (q = 1.78 g/ml) and remains below the water solution. The sample was not covered and the air region was deliberately placed within the active coil volume. A schematic of this sample is shown in Fig. 2.

#### Results

The T<sub>1</sub> values for sodium in the saline solution and for sodium in cartilage were determined as 64.4ms and 18.2ms, respectively. The zero-crossing points in inversion-recovery were determined as 39.24ms and 12.32 ms, respectively.

The pulse sequence in Fig.1 was applied to the free-ordered sodium sample. When  $\tau$ =12.32ms (Fig.3B), the ordered sodium signal (left peak) is totally suppressed, and when  $\tau$ =39.24ms (Fig.3C), the free sodium signal (right peak) is totally suppressed. If  $\tau$  becomes larger, the free sodium signal appears again (Fig. 3D) and when  $\tau$  was large enough, both free and sodium became fully relaxed (plot E in Fig.3).

When the ordered sodium signal was totally suppressed, the magnitude of free sodium signal was at 58.3% of its maximum value. When the free sodium signal was totally suppressed, the magnitude of the ordered sodium signal was 70.6% of its maximum value.

### Conclusion

We demonstrate the inversion recovery method's ability of separating the signals from the free and bound sodium pools by using a mixture of saline buffer and a cartilage sample, where, by changing the relaxation time, we selectively detected the ordered 23Na NMR signal and the free 23Na NMR signal, respectively. The advantage of this method lies in (1) its simplicity, (2) the ability to selectively detect either the ordered or free sodium signal, (3) robustness against flip angle errors, (4) the use of only a small phase cycle, (5) the independence of residual quadrupolar couplings. We will also present results from investigations of the robustness against  $B_1$ ,  $B_0$  inhomogeneities, as well as, the evaluation of long rf-pulse effects with this method [2]. The investigation of other tissue types may also benefit from this method if a sufficient difference in relaxation times

#### References

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Fig.1. Pulse sequence of the Inversion recovery (IR) experiment performed in combination with a gradient spin echo.



Fig. 2. The free-ordered sodium mixture sample used



Fig.3. One-dimensional images obtained using the IR sequence in Fig.1. (A) $\tau$ =1ms, (B)  $\tau$ =12.32ms, (C)  $\tau$ =39.24ms, (D)  $\tau$ =80ms and (E)  $\tau$ =300ms. The signal of ordered sodium is optimally suppressed in (B) and the signal of free sodium is optimally suppressed in (C).