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# Chemically Resolved Nuclear Spin Relaxation

Megan Sly

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Megan Sly
Advisor: Dr. Klaus Woelk
Department of Chemistry
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**Abstract**:

In Nuclear Magnetic Resonance (NMR) Spectroscopy, the excitation of nuclear spins is used to generate spectra of radiofrequency resonance signals. The signal frequencies are typically used to determine the structure of chemical compounds, while the relaxation of the spins to thermodynamic equilibrium provides useful information about the molecular vicinity of a material. When a chemical compound occurs in different molecular environments, a single resonance signal can have multiple relaxation times. To quantify how much material is in how many different environments, a multi-exponential analysis was developed resolving resonance signals and relaxation times in two-dimensional contour plots. Samples of known resonance signals and relaxation times were used to test the implementation of the new technique. For a new type of polymer hydrogels, the contour plots provide information about how much of the gel-forming water is freely moving as bulk solvent and how much is restricted in motion as molecularly bound water.

**Introduction**:

Spectroscopy can be classified as studying the interactions between matter and electromagnetic radiation, where energy is absorbed, transferred, emitted, etc. When subjected to a magnetic field, atomic nuclei that carry a magnetic moment can transition from the nuclear-spin energetic ground state to an excited state. To return to the ground state, the nuclei must lose the excitation energy to their vicinity, i.e., to their immediate chemical environment, often just called the lattice. The transferred energy is observed in a spectrum as a nuclear resonance frequency, and the time progress it takes the nuclei to return to their thermodynamic equilibrium is referred to as nuclear spin-lattice relaxation.

Nuclear Magnetic Resonance (NMR) spectroscopy measures these resonance frequencies in Free Induction Decays (FID).[1] 'Free' meaning that, after the excitation, there is no further outside influence, 'Induction' indicating that NMR is a magnetic induction process, and 'Decay' refers to the first-order relaxation process. To proceed from a recorded FID to frequencies in an NMR spectrum, the FID is subjected to a Fourier Transformation, a mathematical algorithm that changes FID resonances into peaks in an NMR spectrum. Scientists use the location (chemical shift), shape, and multiplicity of peaks in NMR spectra to determine molecular structures of chemicals. The relaxation of the nuclei from excited states to thermodynamic equilibrium is used to identify the immediate environment around the molecules under investigation. As a general rule, nuclei that relax faster toward thermodynamic equilibrium, i.e. nuclei with shorter relaxation times, exhibit broader peaks than those with longer relaxation times. Shorter relaxation times are typically the result of tighter

chemical environments with less degrees of freedom regarding the molecules' mobilities, while freely moving small molecules exhibit longer spin-lattice relaxation times.

Regular NMR spectra are typically collected with 90° excitation radiofrequency pulses, often called observe pulses. In contrast, spin-lattice relaxation times are determined by a series of two-pulse inversion-recovery experiments. In this project, NMR spectra and nuclear spin-lattice relaxation times are resolved simultaneously resulting in two-dimensional contour plots that reveal both the chemical structure as well as the immediate chemical environment of the sample under investigation.

When the new two-dimensional NMR technique was applied to polymer hydrogels, the contour plot revealed different environments for the water molecules based on the resolution of different relaxation times for the same water-molecule resonance frequency. Polymer hydrogels are three-dimensional networks of hydrophilic polymers that can swell in water and hold a large amount of water while maintaining the structure due to chemical or physical cross-linking of individual polymer chains'.[2] The high water content gives the hydrogels a large degree of flexibility. This study shows that water can be located in different chemical environments, such as freely moving water or restricted in motion while attached or bound to the polymer macromolecules.

**Theory:**

Inversion-recovery experiments are typically conducted with the 180° - $\tau$ - 90° NMR experiment.[3] In these experiments, the 180° pulse inverts the macroscopic magnetization of the sample molecules, so that the nuclear-spin population is far removed from thermodynamic equilibrium. The evolution time (evolution delay, symbol: $\tau$) allows the nuclear spins to partially or completely return to thermodynamic equilibrium before a 90° observe pulse is applied to record the remaining magnetization. By recording multiple spectra with varying evolution times, relaxation of the macroscopic magnetization to thermodynamic equilibrium can be followed. After the 180° inversion pulse, the relaxing magnetization ($I_\tau$) follows the mathematical relationship:

$$I_\tau = I_0 (1 - 2\, e^{(-\tau/T_1)}) \qquad [1]$$

where $I_0$ is the macroscopic magnetization at thermodynamic equilibrium, and $T_1$ is the relaxation time constant. Spin-lattice relaxation times can be estimated by adjusting the evolution delay $\tau$ such that the recorded magnetization $I_\tau$ is zero. For $I_\tau = 0$, Eq. [1] reduces to

$$T_1 = \tau / ln(2) \qquad [2]$$

When the same type of material occurs in different molecular environments, a resonance signal at a single location in the NMR spectrum (i.e., at one chemical shift) can exhibit multiple relaxation pathways. The different relaxation pathways result in different relaxation time constants ($T_1$), and a simple analysis according to Eqs. [1] and [2] is no longer possible or accurate. To quantify how much material is in how many different environments, a multiexponential analysis must be used, which resolves relaxation times constants within a predetermined relaxation range. The multiexponential analysis chosen for this project is based on a proprietary, alternating non-negative least-squares (NNLS) algorithm for the optimization of relaxation coefficients. This algorithm was developed by the Woelk group in collaboration with researchers from Argonne National Laboratory near Chicago, IL.

For the optimal performance of the newly developed NNLS algorithm, multiple inversion recovery experiments are needed with the relaxation delay τ varied on an exponentially increasing time scale. For the algorithm to successfully converge, a minimum of 64 - 128 individual experiments with increasing relaxation delays are typically recorded. The individual relaxation delays ($\tau_i$) for these experiments are determined by the formula:

$$\tau_i = T_{1,max}^{(n-i)/(n-1)} \cdot T_{1,min}^{(i-1)/(n-1)} \quad [3]$$

where $i$ is the index of the experiment, $n$ is the number of experiments for the relaxation analysis, and $T_{1,\text{min}}$ and $T_{1,\text{max}}$ determine the minimum and maximum time constants in the predetermined relaxation range, respectively.

**Experiment and Results:**

The polymer hydrogel samples used in this project were provided by the research group of Dr. T. Schuman. They were generated directly in 5-mm NMR sample tubes. The sample tubes were inserted into the magnet of a 200-MHz Bruker AVANCE DRX wide-bore NMR spectrometer. 128 inversion-recovery NMR experiments were conducted with relaxation delays determined by Eq. [3] using the following parameters:

- Number of inversion-recovery experiments: $n = 128$
- Index of inversion-recovery experiments: $i = \{1, 2, ..., n\}$
- Minimum relaxation time constant: $T_{1,\text{min}} = 0.01$ s
- Maximum relaxation time constant: $T_{1,\text{max}} = 11.0$ s

The 128 spectra of the inversion-recovery experiments (32 k data points each) were reduced to 128 data points each by integration over 256 consecutive data points in the spectra, generating reduced resolution spectra suitable for the NNLS algorithm. The resulting 128 x 128 matrices of chemical shift vs. relaxation-delay data were processed by the alternating NNLS algorithm, providing 128 x 128 matrices of relaxation coefficients vs. chemical shift data. To generate the desired contour plots of relaxation time constants vs. chemical shift, the 128 x128 data matrices were entered into the spreadsheet of the scientific graphing software SigmaPlot (Version 14) and processed accordingly. Figure 1 shows the resulting contour plot for a poly (amino methacrylate) hydrogel.

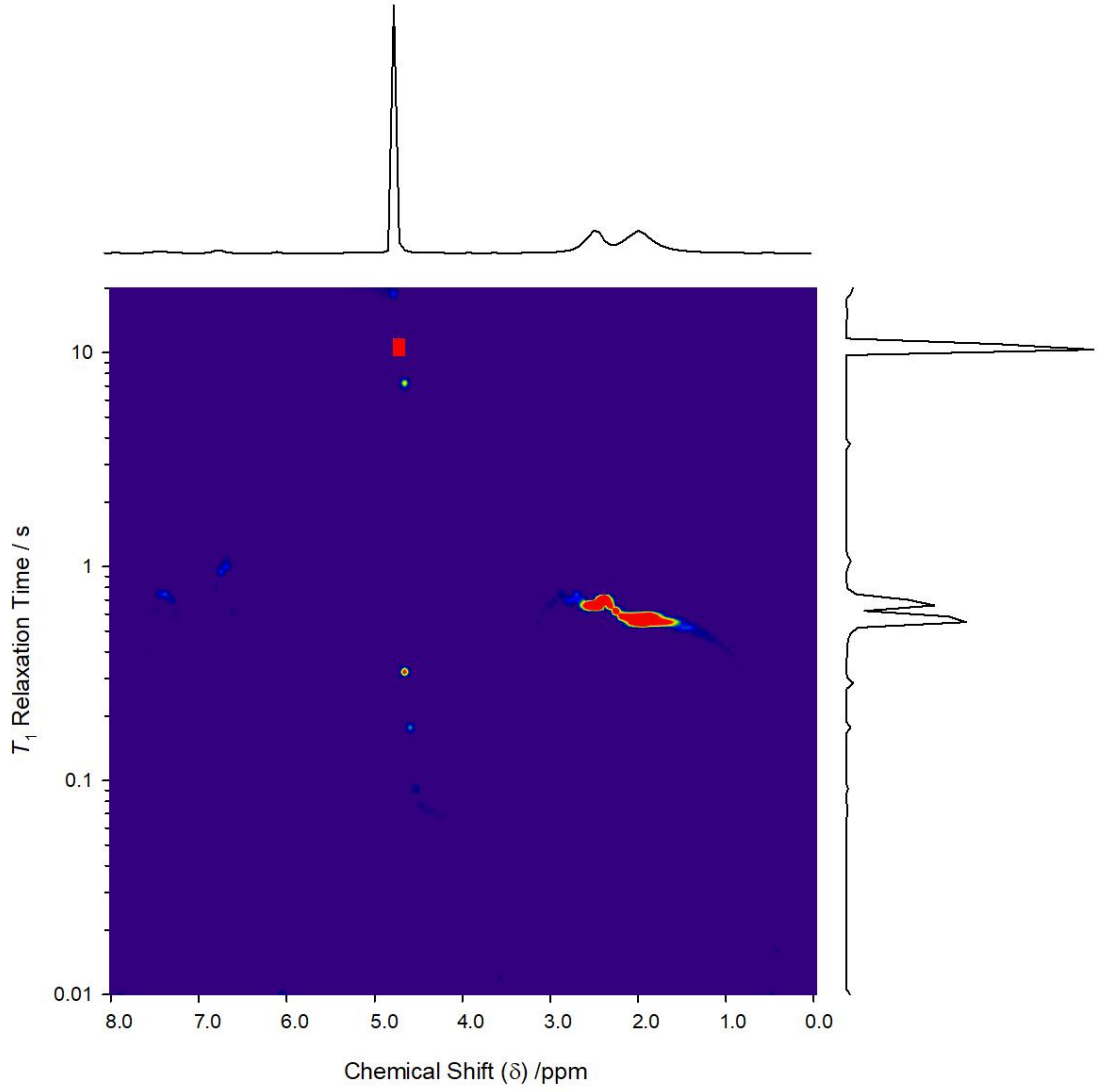


**Fig. 1.** Chemical shift vs. relaxation time contour plot of a poly (amino methacrylate) hydrogel. The colored signals in the plot represent relaxation times for the respective chemical-shift peaks. The

resonance spectrum of the hydrogel is placed on top of the contour plot while the intensities of the relaxation signals are represented in the plot to the right.

The contour plot of Fig. 1 reveals that the water molecules (chemical shift of 4.5 ppm) reside in two different chemical environments (relaxation times of 10 s and 0.35 s). The relaxation time of 10 s is identified with bulk water that is freely moving, while the reduced relaxation time of 0.35 s indicates motionally substantially restricted water molecules such as bound to the polymer chains. A further, detailed quantitative analysis (Fig. 2) reveals that the hydrogel sample under investigation has 6.6% of bound water molecules while 93.4% are behaving like non-viscous, liquid water. The fact that the relaxation time signals in the contour plot are very distinct indicates that there is no exchange between free and bound water molecules on the measured relaxation time scale.

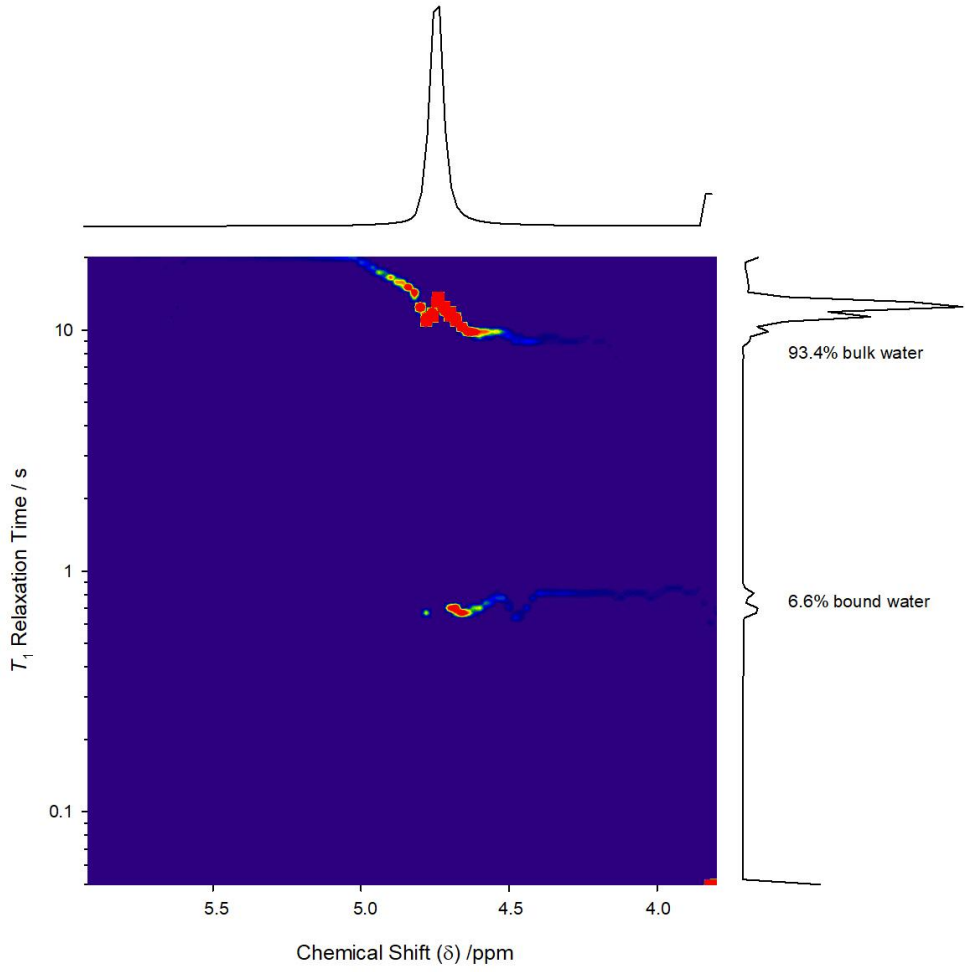


**Fig. 2.** Zoomed view of the chemical shift vs. relaxation time contour plot of a poly (amino methacrylate) hydrogel for the region of the water resonance at 4.5 ppm. Quantification of the

relaxation signals at 4.5 ppm reveals 6.6% of the water molecules bound to the polymer matrix, while 93.4 % appear to be freely moving throughout the hydrogel.

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**References:**

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