Portable Time-Domain NMR: A rapid method for detecting changes in complex pharmaceutical materials and formulations.

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ABSTRACT

The purpose of this study was to evaluate the ability of time-domain NMR (TD-NMR) to detect differences between pharmaceutical materials, such as antibodies. TD-NMR measures the differences in molecular mobility between components in a solution as reflected in the longitudinal (T1) and transverse (T2) relaxation times of protons. This study demonstrates that TD-NMR techniques can distinguish between different biological compounds and can distinguish biological compounds at different concentrations. Experiments using these techniques can be conducted using small, dedicated TD-NMR instruments; low-field bench-top instruments; as well as the high field instruments commonly used at AbbVie. For this study, a portable TD-NMR instrument, the WaveGuide Formμla™, was used to measure the T2 relaxation times.

TD-NMR has been particularly successful with identity testing applications. The technique analyzes fast chemical exchange between water and exposed NH and OH protons of amino acid side chains in the folded protein structure unique to each biologic. Analysis of dominant features in folded proteins in solution can be exploited for other types of biopharma assets as well as for authentication, forensics, and supply chain integrity where other spectroscopic or chromatographic testing techniques cannot detect a difference. In addition, TD-NMR has been used to determine the water content of lyophilized proteins and the aggregation of proteins in solution. For small molecule applications, TD-NMR can detect if solvents are received neat or tainted with moisture, impurities, or denaturants. The results presented in this poster demonstrate a preliminary evaluation of TD-NMR for testing applications relevant to the manufacture of antibody pharmaceuticals.

INTRODUCTION

Experiments evaluating sample composition using time-domain NMR (TD-NMR) are commonly based on directly fitting a time-domain decay signal to determine the decay time constants. Such experiments can distinguish between components (phases or compounds) that have different molecular mobilities. The relaxation time constants, T1 and T2, are physical properties of the sample and are sensitive, e.g., to molecular mass, viscosity of a liquid sample, or "rigidness" of a solid sample. T1 (longitudinal relaxation time) is the time constant that determines the rate at which excited protons return to equilibrium, which is a measure of the time taken for protons to realign with the applied magnetic field. T2 (transverse relaxation time) is the time constant that determines the rate at which excited protons decohere, which is a measure of the time taken for protons to lose phase coherence in the plane perpendicular to the applied field.[1]

TD-NMR can operate at low magnetic fields, allowing the use of permanent magnets, which are simple, inexpensive, and do not need extensive cooling by liquid gases. Research at Harvard University resulted in a novel way to miniaturize TD-NMR relaxometry and NMR spectroscopy, leading to the development of the WaveGuide Formμla™ instrument used in this study.

TD-NMR has long been used in the food industry to study fat content, fat crystallization, total fat, oil content, moisture and droplet size in emulsions. This technique has enabled rapid and accurate testing in quality labs throughout the food industry. The success of these applications has encouraged the development of applications in the petrochemical and pharmaceutical industries.

Protein Studies

TD-NMR has been used to demonstrate presence of monoclonal antibody (mAb) aggregates generated by different stresses.[2] In this study, water proton transverse relaxation rate R2(1H2O) measurements were used to demonstrate the potential of TD-NMR as a quantitative and noninvasive analytical tool for characterizing protein aggregates in biopharmaceutical formulations. Based on this work and others, proof of concept investigations were initiated to use TD-NMR for rapid identity testing for antibodies using a portable TD-NMR device.

Current mAb identity testing methods include peptide mapping, which is selective, but comparatively time-consuming and expensive.

OBJECTIVE

Evaluate the capability of TD-NMR as an analytical tool to discriminate between antibodies for identity testing.

METHODS

Antibody Identification

Initial study for pharmaceutical biologics:

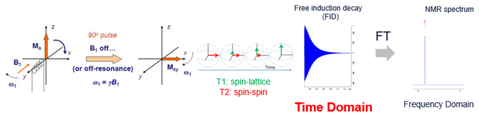
- ❑ Experiment A: Evaluate the capability for a reliable analysis metric that can discern different antibody species (20 unique substances).
- ❑ Experiment B: Measure the effect of antibody stability on the T2 measurement:
  - Immediate analysis after -80°C thawing
  - Analysis after 24 hours at 25°C
  - Forced aggregation at 90°C (15 minutes)

Procedure:

- ❑ For each sample, perform T2 measurement on WaveGuide Formμla™ TD-NMR instrument.
- ❑ Fit the measured T2 relaxation decays with single- and bi-exponential decay models and apply Laplace inversion analysis.
- ❑ Analyze the corresponding decay times and component amplitudes.

The WaveGuide Formμla™ portable TD-NMR
- Little to no sample preparation required
- Sample analysis take only minutes
- Less than 30μL of sample typically used per test

T2 measurement by Time Domain NMR[1]

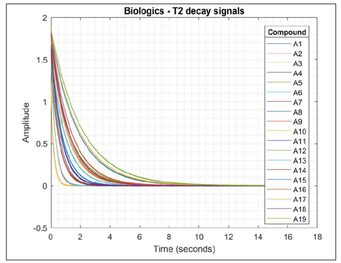


T2 is dependent on:
- Mobility of nuclear species, the presence of macromolecules.
- T2 increases with decreased molecular size and increased molecular mobility.

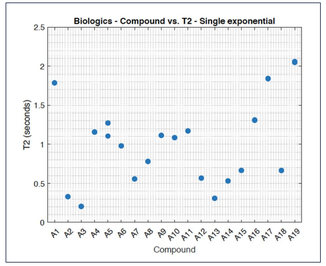
	Time Domain NMR	Frequency Domain NMR
Fourier Transform	No	Yes
Outcome	Numeric	spectrum
T2 measurement on proton	Bulk method	Target specific
Instrumentation	Benchtop, uNMR	High-field, benchtop

RESULTS: TD-NMR Analysis of 20 Unique Biological Compounds

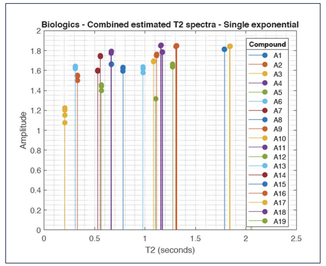
Three 15 μL aliquots were drawn from each sample vial and tested at a temperature of 25°C unless noted



Graph 1 (above): T2 decay relaxation times from all the 20 unique samples.

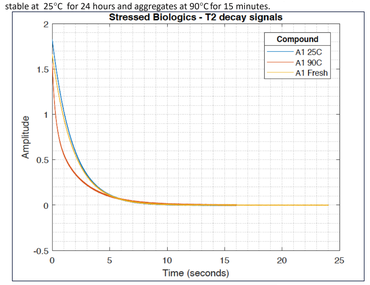


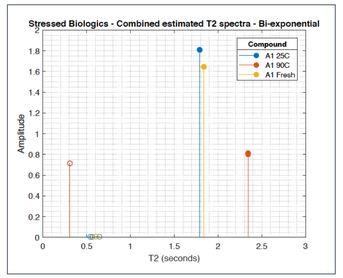
Graph 2 (above): T2 value of the single exponential component had a range of 0.256 (sec) to 2.014 (sec). All compounds displayed a unique single component exponential fit.



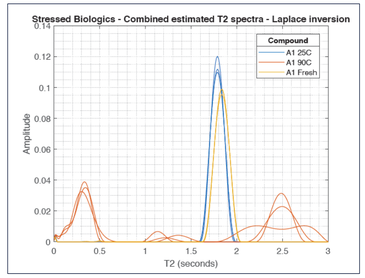
Graph 3 (above): Single component exponential fit graphed to showing unique compound amplitudes and T2 values.

Graph 4 (below): T2 relaxation decay curve for stressed sample A1. Representative Sample A1 is stable at 25°C for 24 hours and aggregates at 90°C for 15 minutes.

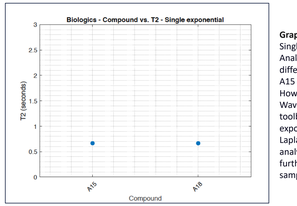




Graph 5 (above): Stressed Sample A1 Bi-Exponential Fit

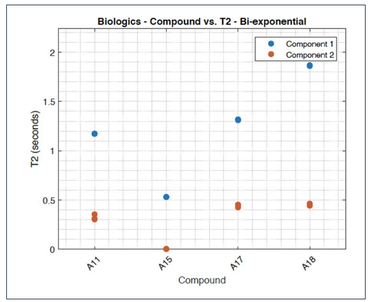


Graph 6 (above): Sample A1 Laplace inversion representation. **Graphs 4, 5 and 6** illustrate the potential to investigate sample A1 decomposition pathway.

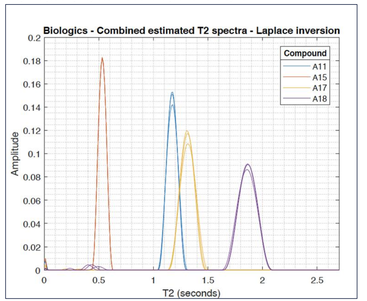


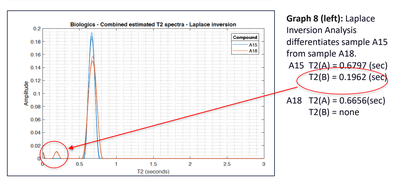
Graph 7 (left): Single exponential Analysis does not differentiate sample A15 from sample A18. However, the WaveGuide TD-NMR toolbox allows for bi-exponential and Laplace Inversion analyses that can further distinguish the samples.

Graph 9 (below): This sample set was difficult to qualitatively analyze utilizing Raman see Graph 11. Early results suggest that TD-NMR technique can quantitatively distinguish between different species with different concentrations.



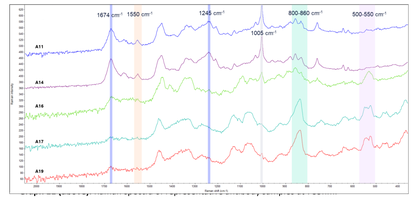
Graph 10 (below): Laplace Inversion analysis view of Graph 9.



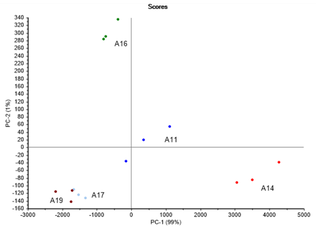


Graph 8 (left): Laplace Inversion Analysis differentiates sample A15 from sample A18.
A15 T2(A) = 0.6797 (sec)
T2(B) = 0.1962 (sec)
A18 T2(A) = 0.6656(sec)
T2(B) = none

TD-NMR: Correlates to Raman Investigation



Graph 12 (below): Principal Components Analysis of representative Raman profiles, 785nm.



- ❑ Both TD-NMR and Raman Spectrometry
  - Selective
- ❑ TD-NMR
  - Handles small volume liquid samples easier
  - Less difficult to interpret results
  - Likely can execute selectivity for larger data sets

CONCLUSIONS

- TD-NMR has demonstrated excellent discrimination capability for use in antibody identification using an innovative portable instrument. Further studies with this instrument are warranted.
- Discrimination of antibodies has been demonstrated in using TD-NMR and Raman Spectrometry.
- All studies were proof of concept – larger data sets are needed for further evaluation.

REFERENCES

1. https://case.edu/med/neurology/NR/MRI%20Basics.html
2. Anal. Chem. 2019, 91, 6, 4107–4115
3. Proc. Nat. Acad. Sci. (PNAS) 111, 11955 (2014)

DISCLOSURE

- Marcus Semones, Jeff Bernstein, Massimiliano La Colla and Wendy Graham-Coco, are employees of WaveGuide Corporation.
- Gregory K. Webster, Steven J. Doherty, Cassie Yang, and Sankaran Anantharaman are employees of AbbVie.
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