Multiplatform Benchtop NMR Inter-Lab Study of Model Liquid Dosage Forms of Pharmaceutical Products

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Background

Benchtop NMR has proven to be a useful tool in the food and oil industries. In the pharmaceutical and biotech industries, high-field NMR is regularly used as analytical tool in R&D but is incompatible with manufacturing. Benchtop NMR is compatible with manufacturing, but its potential has yet to be realized. Some groundwork in this area indicates that the transverse relaxation rate of the water proton signal, $R_2(^1H_2O)$, quite usefully, can detect changes in drug concentration (1), protein aggregation content (2), or freeze/thaw of vaccines (3), and can serve as a contact-free, in-line process analytical technology (PAT) (4). To transfer this work to the broader community, some standardization is needed, hence this inter-lab comparison on the benchtop NMR $R_2(^1H_2O)$ measurement.

Many pharmaceutical companies are racing to produce safe, and effective vaccines against SARS-CoV-2 for billions of people. The aggressive timeline for producing and massively scaling-up manufacturing of vaccines brings to focus the need for quality assurance technologies (5). Publicizing the results of these pilot studies involving major benchtop NMR manufacturers will inform the pharmaceutical industry that the implementation of benchtop NMR instruments could save time, money, and lives if implemented at various check points in the pharmaceutical pipeline.

Scope of work

In this study, we will assess low-field benchtop water NMR methods for characterizing model liquid dosage forms of pharmaceutical products. The general plan is to mail a series of samples to each partner; partners then measure the transverse relaxation time of water ($T_2(^1H_2O)$) and send the data back to us for analysis. The goal is to validate that, across platforms and different instruments, these $T_2(^1H_2O)$ measurements are reliable, reproducible, and informative methods for characterizing pharmaceutical products.

Study Protocol

Each partner will be mailed the same 25-30 samples sealed in 15 mm O.D. glass vials along with instructions for sample storage, for conducting the measurement, and for reporting the data to NIST/UMB.

Sample Contents

Liquid dosage forms of pharmaceutical products include solutions, emulsions, or suspensions. The samples in this study will model liquid dosage forms of pharmaceutical products. The contents of the samples will be some combination of the following: NIST monoclonal antibody (NISTmAb), NIST ethylene tetrafluoroethylene (ETFE) particle suspension, aluminum hydroxide gel suspension, aluminum phosphate gel suspension. The samples will be labeled a number or letter so that the sample contents will be blinded. As with most liquid dosage forms, the main component of all the samples will be water. Each of the 25-30 sample vials will be approximately 0.5 mL in volume. A vial of Ferrlecit®, an iron carbohydrate drug product, will be supplied as a standard, along with the expected $T_2(^1H_2O)$ of the drug product at 25°C. All samples are non-hazardous, and the sealed sample vials will be mailed to all partners.

Sample Storage

Upon receipt of the shipment of samples, each sealed vial in the carton should be visually inspected for evidence of vial freezing (*i.e.*, ice formed inside the vial), and then the samples should be swiftly placed into a refrigerator (2–8 °C). All samples should be stored at ~4 °C whenever they are not being measured.

Upon receipt of the single vial of Ferrlecit®, which will be shipped separately, visually inspect the vial for signs of physical damage to the vial or contents (*e.g.*, cap dented, septum pierced, ice formed). Ferrlecit® should always be stored at 20-25 °C, or room temperature.

Sample Handling

At least 40 minutes before measuring samples, equilibrate the sample vial at the measurement temperature. Handle each vial by the aluminum cap to prevent fingers from warming the sample. Gently invert the sample 20 times (back and forth), trying not to produce bubbles and/or foam. Set the sealed vials on a benchtop surface, away from sunlight, until the samples to be measured equilibrate to the room temperature.

If the temperature of the room is different from the measurement temperature of the NMR, consider equilibrating the samples individually in the NMR for 40 minutes prior to a measurement, or placing samples in a water bath calibrated to the set temperature of the NMR instrument.

After the samples are temperature equilibrated and experimental parameters are set, retrieve the sealed sample vial (from the benchtop NMR bore, water bath or benchtop), grasp at the aluminum cap, and invert 20 times gently, again trying not to produce bubble and/or foam, and then place the sample into the instrument for acquisition of the measurement. This will ensure that suspensions are fully suspended for the $T_2(^1H_2O)$ measurement of the sample.

Data Collection

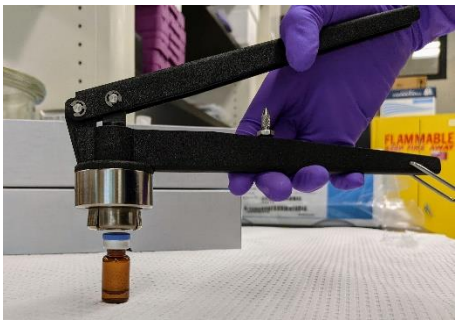
Harnessing the high concentration of the water component, each partner will measure the transverse relaxation time of the water protons, $T_2(^1H_2O)$, of each of the samples using a CPMG pulse sequence. The sealed sample vials are to be measured directly in the benchtop NMR instrument. The samples should be run at a single temperature between 25-30 °C, with 28 °C being optimal for this study. The 25-30 sample vials for analysis should be measured at the exact same temperature using the same primary instrument and should have the same inter-pulse delay (*e.g.*, 500 µs). Additional benchtop NMR instruments may also be used to measure all or a subset of the samples. If these additional measurements are done, the additional data sets should be reported with parameters and experimental details specific to the benchtop NMR instrument that was used.

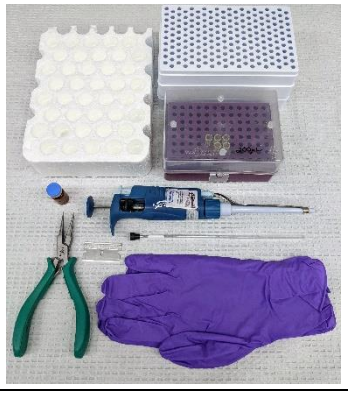
Following the measurement of the sealed vials, each partner will transfer samples to a standard 4 mm or 5 mm NMR tube (see *Sample Transfer*) and again measure the $T_2(^1H_2O)$ of each sample.

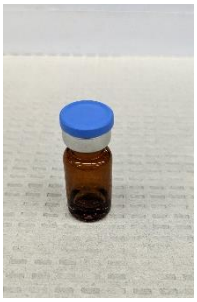
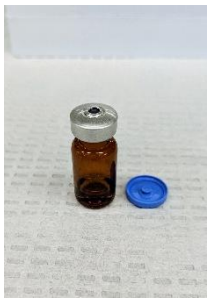
Sample Transfer

If your benchtop NMR instrument can measure the materials in the provided sealed, unopened vials, complete the measurements with the sealed, unopened vials first, and then transfer samples from the vial to a standard 4 or 5 mm NMR tube. If possible, collect data using the 4 or 5 mm NMR tube on the same benchtop NMR instrument as was used for the sealed vials (see *Data Collection*).

In the absence of a 13 mm (cap size) hand decrimper decapper, the following is a protocol for transferring liquid sample from the sealed vial into an NMR tube. If an alternative method is used, the most important aspect of transferring samples is to gently invert the sample just before pipetting (minimizing bubbles) to ensure that suspensions are fully suspended. Keep all sealed sample vials at 4 °C until each vial is ready for cap removal and sample transfer to minimize time at room temperature.



<u>Materials:</u> ▪ Gloves ▪ Pipette and tips ▪ Razor blade or box cutter ▪ Needle-nose pliers ▪ Vial rack ▪ Sealed sample vials ▪ NMR tubes (5 mm or 4 mm) with caps	

<table>
<tr>
<td>Transfer Procedure:
1) Clean and clear a counter space. Place vials in rack.
2) Prepare clean and dry NMR tubes and caps for transfer.
3) Wear gloves.
4) Pop off colored cap.</td>
<td>   </td>
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<tr>
<td>5) Use a razor blade (taking lots of care not to nick yourself) to press the blade into the aluminum rim to create a perforation line (without pressing too much into the grey rubber septum).</td>
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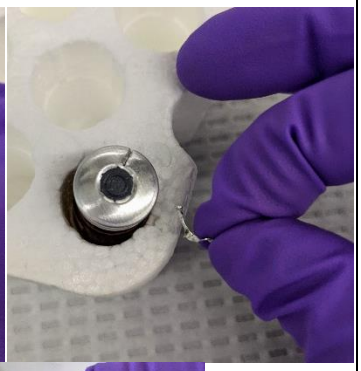
<td>6) Once a perforation has been started, use the short edge of the razor blade (or a less sharp utensil) to pull off the inner ring at the top of the aluminum seal. Continue to pry the outer aluminum rim from the top.</td>
<td>    </td>
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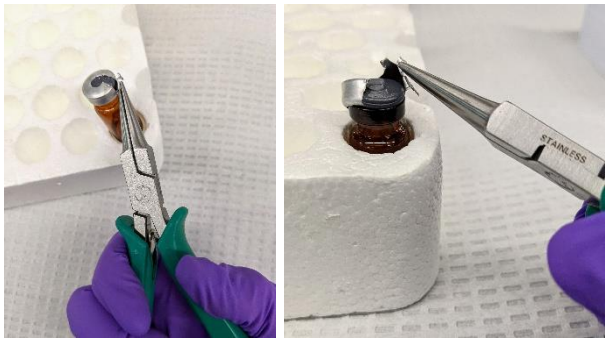
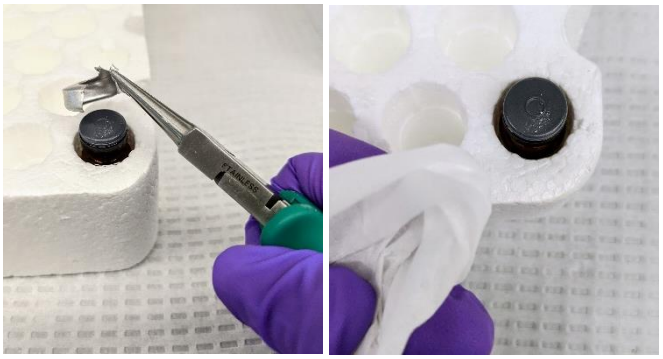
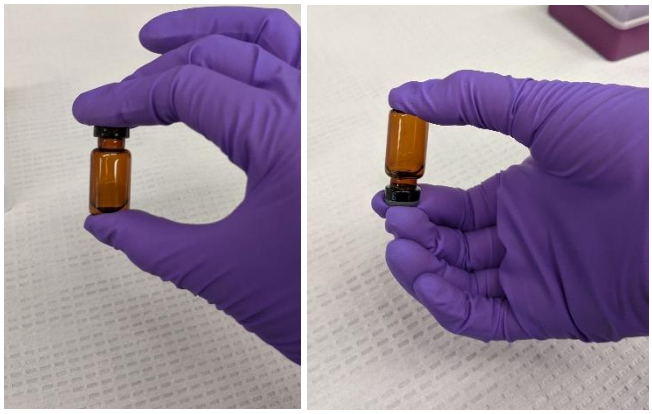
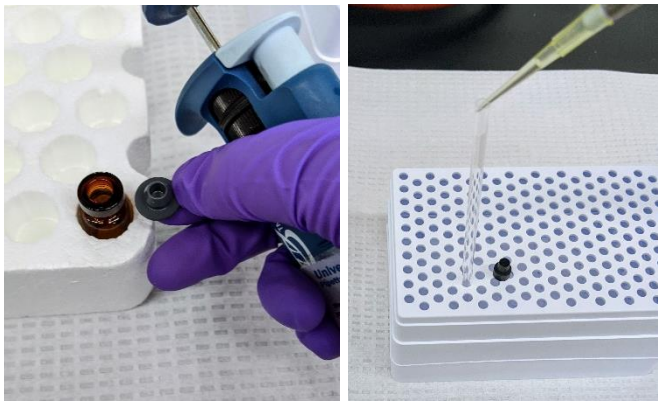
7) Once an edge has been pried up enough, use needle-nose pliers to pull off the seal without cutting fingers. Non-dominant hand can hold vial in place to prevent from tipping over.	
8) Keep septum seal in place while aluminum seal is completely pulled off. Wipe off aluminum remnants from septum.	
9) Hold the vial in hand, firmly pressing the septum at the top of the vial, and invert vial 20 times to resuspend suspensions. 10) Tap or lightly flick the vial so any liquid from underneath the cap slides down to the bottom of the vial.	
11) Set vial in rack. Remove septum. Pipette 0.5 mL liquid sample from vial to NMR tube. Check cap for remaining liquid and transfer this liquid too. Cap NMR tube. 12) Store NMR tubes at 4 °C.	

Samples in 4 or 5 mm NMR tubes should be brought to the measurement temperature and resuspended prior to data collection in the same way as the sealed vials (see *Sample Handling, Data Collection*).

Data Analysis

Once the data has been collected, partners will send to the following data and information to the IBBR Team:

1) Instrument(s) specifications (*e.g.*, magnetic field strength, bore size, temperature inside the cavity).

2) Acquisition parameters.

3) Simple text format of the data (time, real, imaginary) for extracting the $T_2(^1H_2O)$ (*e.g.*, in an excel spreadsheet).

4) Extra information about how the experiment was conducted with respect to the pulse sequence, instrument parameters, sample preparation, and sample transfer.

The simple text format of the data will be anonymized by assigning each data set a generic name (*e.g.*, 'Lab 1', 'Lab 2', etc.). The published data will be presented without associating the data with the partners. The anonymized data will be made available to all partners.

Use of Data

All data collected will be freely available to all partners in the study. Public dissemination of the results from the inter-laboratory should represent a consensus of the partners which will be arrived at through group forums (*e.g.*, conference calls, emails) mediated by UMB/NIST. This consensus must be agreed upon before the first public presentation.

Upon publication, all data will be made publicly available.

Reporting

All data will be reported at selected scientific conferences and in a notable peer-reviewed journal.

Benefit to the Partners

Each partner will have the benefit of contributing to an exercise that will set standard methods and protocols for the application of benchtop NMR for the assessment of pharmaceutical products. One desired outcome is for NMR vendors to consider their benchtop NMR designs for implementation in the entire pharmaceutical sector, including manufacturers, distributors, regulators and end-users, and for this study to lead to a larger follow-on study with many more partners across academia and the pharmaceutical sector.

References

1. Briggs, K.T., M.B. Taraban, W. Wang, and Y.B. Yu. 2019. Correction to: Nondestructive Quantitative Inspection of Drug Products Using Benchtop NMR Relaxometry-the Case of NovoMix(R) 30. *AAPS PharmSciTech*. 20:214.

2. Taraban, M.B., R.A. DePaz, B. Lobo, and Y.B. Yu. 2017. Water Proton NMR: A Tool for Protein Aggregation Characterization. *Anal. Chem.* 89:5494–5502.

3. Briggs, K.T., M.B. Taraban, and Y.B. Yu. 2020. Quality assurance at the point-of-care: Noninvasively detecting vaccine freezing variability using water proton NMR. *Vaccine*. 38:4853–4860.

4. Taraban, M.B., K.T. Briggs, P. Merkel, and Y.B. Yu. 2019. Flow Water Proton NMR: In-Line Process Analytical Technology for Continuous Biomanufacturing. *Anal. Chem.* 91:13538–13546.

5. Parrish, M. 2020. Coronavirus vaccines: The billion dose dash. *PharmaManufacturing*.