# Long-Term Compound Storage in Acoustically Enabled Tubes: Theory and Experiment

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## Introduction

Acoustic transfer of compounds between microplates by Echo® liquid handling instruments has facilitated high-throughput drug discovery at a reduced cost. Until now, these workflows used Echo® qualified microtiter plates with either 384 or 1536 wells. We have developed an acoustic tube in a 96-tube format rack that offers modular placement of tubes at each location. Each tube can be individually picked-and-placed or individually opened-and-closed with associated automation. The tubes can be accessed one at a time, all together, or in any combination. Transferring and storing compounds in DMSO from any rack position has been enabled. We evaluated the effect of fill volume and number of decapping cycles on the overall tube performance in terms of maintaining DMSO sample integrity.

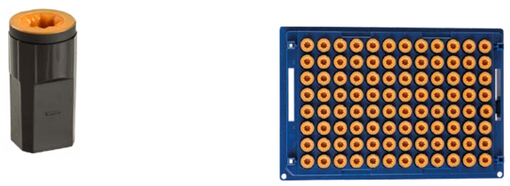


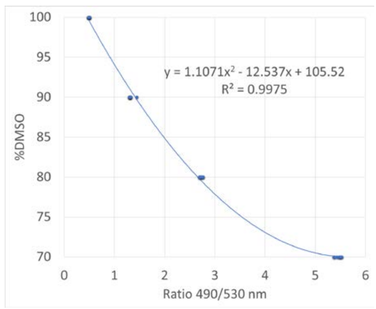
**FIGURE 1:** (left) Acoustic tube with screw-on cap and (right) 96-tube rack.

## Background

Acoustic droplet ejection (ADE) eliminates disposable tips and streamlines liquid handling processes. Sample management and long-term compound storage is important to maintain precious libraries and reduce waste. However, until now, long-term sample storage tubes have been incompatible with Echo acoustic dispensing technology. We developed an Echo qualified acoustic tube that is compatible with Echo ADE technology.

1. *The differentiation of these Echo qualified acoustic tubes is the combination of long-term storage and ADE compatibility.*
2. *Tubes can be opened-and-closed hundreds of times to extract small volumes of fluid without compromising the seal integrity or compound viability.*

We designed a study to measure the key metrics related to sample integrity or long-term storage of compounds in DMSO. To assess the concentration of DMSO we utilized a correlation between absorption at 490 and 530 nm with binary water-DMSO concentration. The ratiometric absorption correlation is valid when using 0.15 M sodium fluorescein in DMSO.



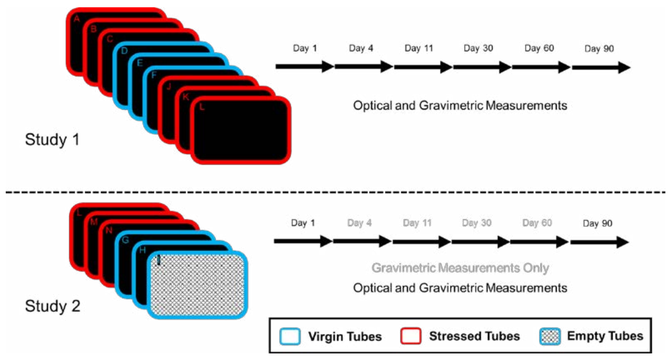

## Materials & Methods

### Absorbance and Gravimetric Measurements

Optical absorbance measurements were made using the Trinean DropSense96 (Unchained Labs) instrument with a standard 96-channel microfluidic chip. At each time-point a 2 µL aliquot was transferred from the tubes to the Trinean chip.

Racks and tubes were individually weighed at each time-point.

For the duration of the experiment, tube racks were stored in a 10% relative humidity (RH), 20 C environment.




## Results

### Stressed vs. Virgin Caps/Tubes

A direct comparison of stressed and virgin caps/tubes reveals there is no statistical difference ($p<0.05$) over the first 90 days of testing. Analysis are separated by tube weight, which tracks evaporative loss, and %DMSO, which tracks the solvent concentration within the tubes.

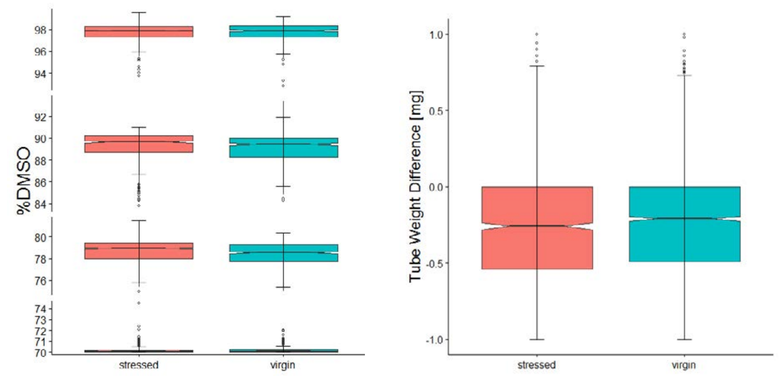



**FIGURE 2:** Boxplot of solvent concentration, grouping multiple time-points and delineating whether the tube has been stress cycled before filling or remained in its virgin state (left). Boxplot of tube weight differences (month-to-month) for stressed and virgin tubes (right).

### Fill Volume Effects

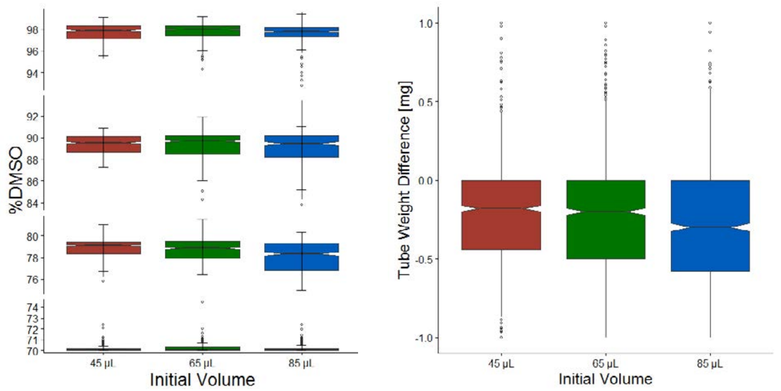
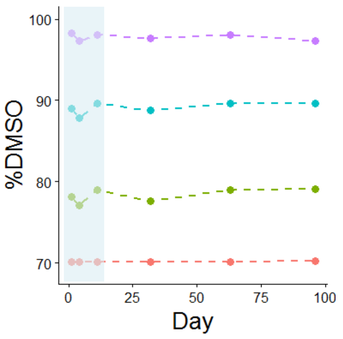



**FIGURE 3:** Boxplot of solvent concentration, grouping multiple time-points and delineating whether the tube has initially filled 45, 65, or 85 µL (left). Boxplot of tube weight differences (month-to-month) for tubes initially filled with 45, 65, or 85 µL of solution (right).

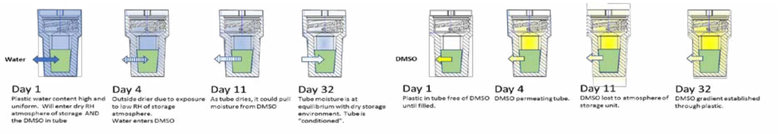
### Plastic Conditioning Period

We designed a study with the expectation that DMSO concentration changes would follow a log time-scale. This fine resolution in the initial time-points revealed a transient drop in %DMSO due to absorption and desorption of DMSO and water by the tube plastic.




| Plastic Weight | 353 mg |
|---|---|
| Plastic Moisture Content | ~0.1% (literature) |
| Max Weight of Water in Plastic | 0.353 mg |
| Nominal Fluid Volume & Weight of 100% DMSO | 70 µL / 77.65 mg |
| Final DMSO% for 100% DMSO If All Plastic Moisture Entering Nominal Fluid Volume | 100 – (0.35/78) = 99.5% |

**FIGURE 4:** Average %DMSO measured at each time-point, grouped by initial DMSO concentration within each tube. The initial dip in concentration is reasonably attributed to a transient conditioning of the plastic.




## Tube Lifetime Model

### Experimental Conditions

- Storage equilibrium: 94% DMSO
- Workcell equilibrium 80% DMSO
- Maintain >90% DMSO for 10 years

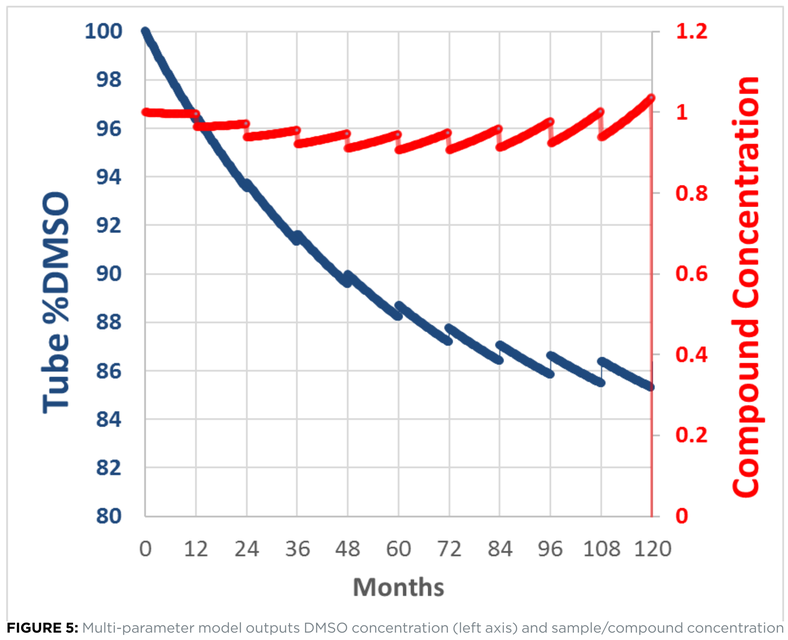



**FIGURE 5:** Multi-parameter model outputs DMSO concentration (left axis) and sample/compound concentration (right axis).

## Discussion & Conclusions

- We expect the DMSO concentration to follow an exponential decay and the 90-days data was fit to an equation of $y = A * \exp\left(-\frac{t}{B}\right) + C$
- At these environmental conditions, C, the equilibrium DMSO concentration is 83%, as measured
- We developed a comprehensive model that incorporates many parameters including the half-life time constant for DMSO hydration in sealed acoustic tubes, B, and the rate of evaporation from sealed tubes – equal to the weight differential weight over time.
- Additionally, we have incorporated an annual addition of 1-3 µL of 100% DMSO to reconcile the loss due to DMSO loss.
- Liquid dispensing from tubes via ADE requires opening and closing of the tubes. This time outside the storage conditions and exposed to the workcell environment is also captured in our model.
- The lifetime of a tube is dependent on these parameters.
- *Using a standard workflow of weekly 75 nL dispenses, the above model indicates tube storage achieves >90% DMSO and consistent compound concentration for 10 years.*

## Aknowledgements

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