



Adaptive Focused Acoustics

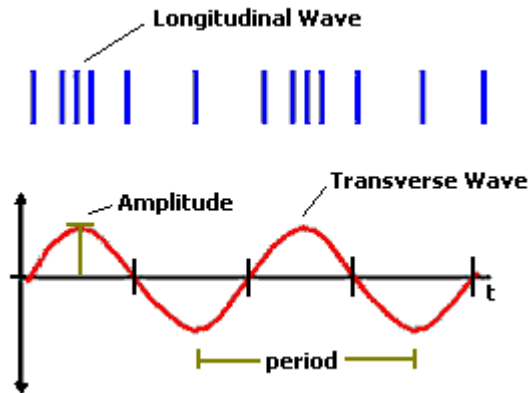
Overview

- Introduction
- Beginnings
- Collaboration
- Technology
- Experiments
- Benefits
- Next Steps
- Other Applications
- Contributors



Introduction

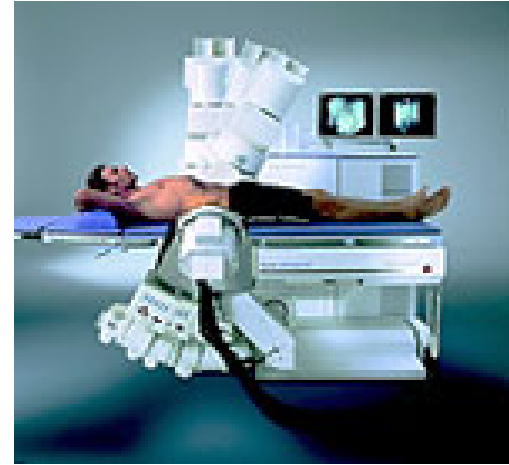
Acoustic waves and their properties have been utilized in research for decades. Operations such as sonication, ocean exploration, warfare, etc, apply acoustic waves.



It wasn't until recently that acoustic waves were used at much shorter wavelengths allowing focused applications.

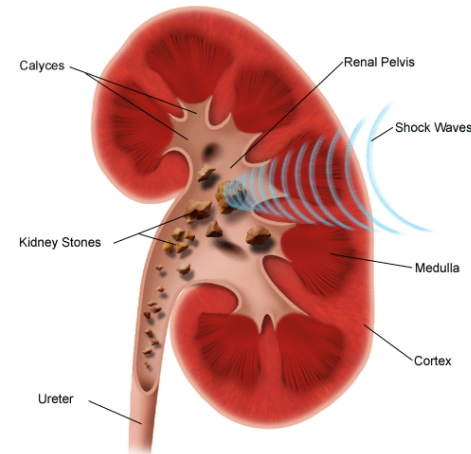
Introduction

Introduced in 1980,
Extracorporeal Shockwave
Lithotripsy (ESWL) treatments
apply non-contact acoustic
properties to break down
kidney and gall stones.

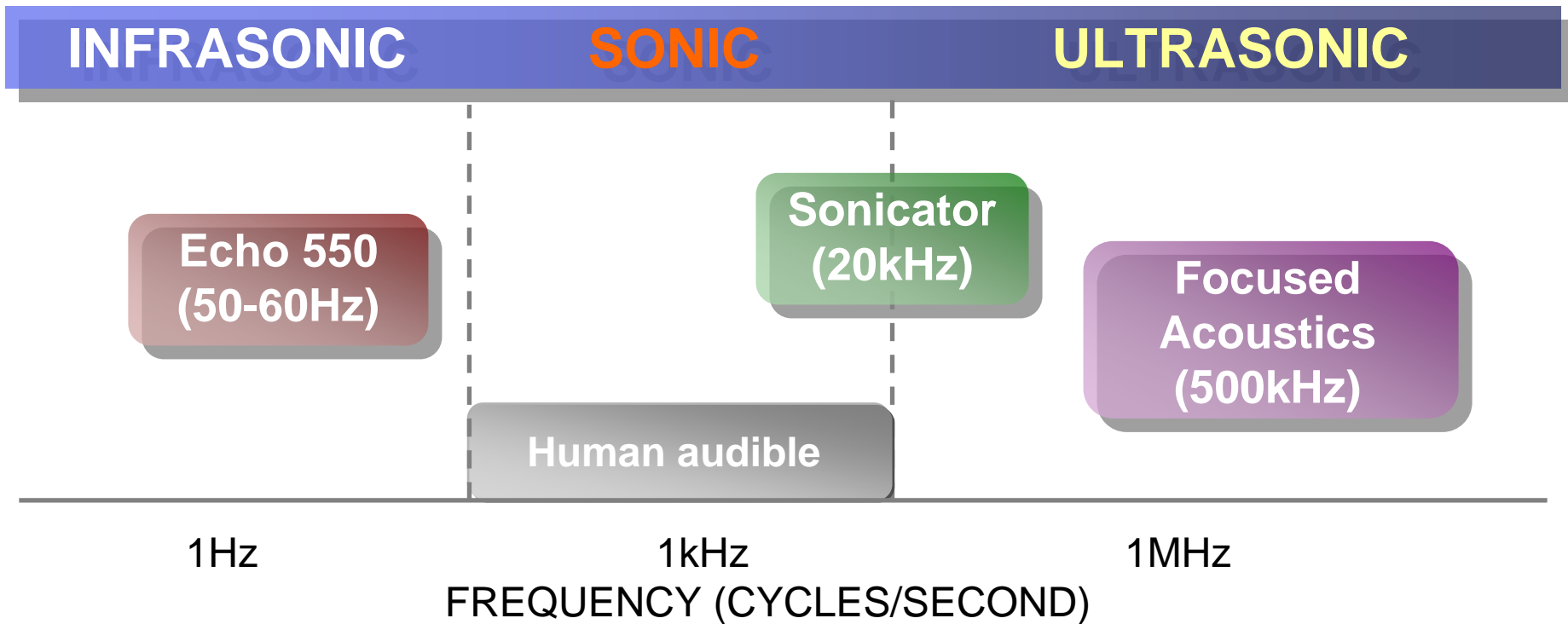


Lithotripsy

Ultrasound imaging also uses
related technology.



Introduction: Acoustic Frequency



Introduction: Acoustic Wavelength

Sonicator

Focused Acoustics

Sonic

Ultrasonic

1m 100mm 10mm 1mm 100um 10um
WAVELENGTH (IN WATER)

Higher frequencies, mean shorter wavelengths and therefore focused energy.

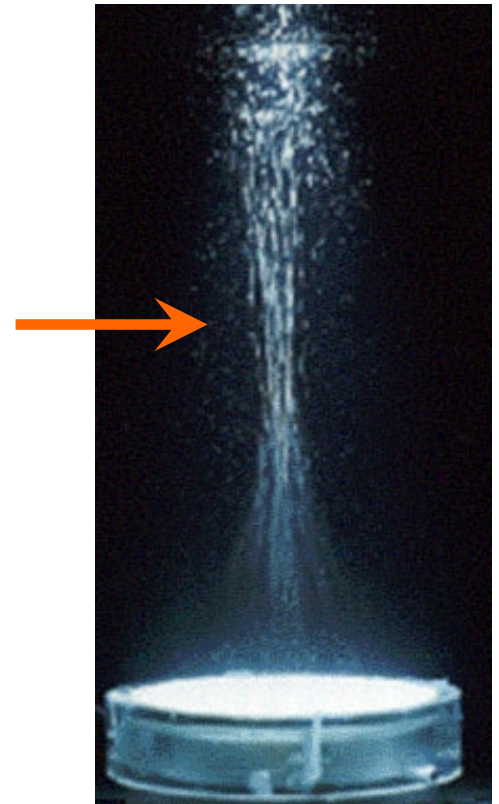
Beginnings

Formed in 1999, Covaris, Inc specializes in Adaptive Focused Acoustics or AFA for biological and chemical applications.

Covaris
tools for analytical sciences



By emitting controlled focused acoustic waves, their systems are able to mix, homogenize, dissolve, and thaw samples with ease.



Beginnings

With the push towards a more high throughput, automated environment, sample management saw a need for a more effective dissolution method.

Dissolution methods include:

Vortex



- Manual
- Isothermal
- Vials must be capped
- Gum/Glue dissolution: Slow process
- Not easily integrated into automation
- Non-flexible and container specific
- Long thaw time

Wet Sonication



- Manual
- Non-Isothermal
- Vials must be capped
- Gum/Glue dissolution: Slow process
- Not commonly integrated
- Non-flexible and container specific
- Short thaw time, but produces heat resulting in potential degradation

Collaboration

GSK, Covaris, and KBiosciences came together to develop a way to reduce dissolution time and variability, standardize methods, and minimize freeze/thaw cycles.



Collaboration

The answer was an automated system incorporating pick and place robotics and Adaptive Focused Acoustics.

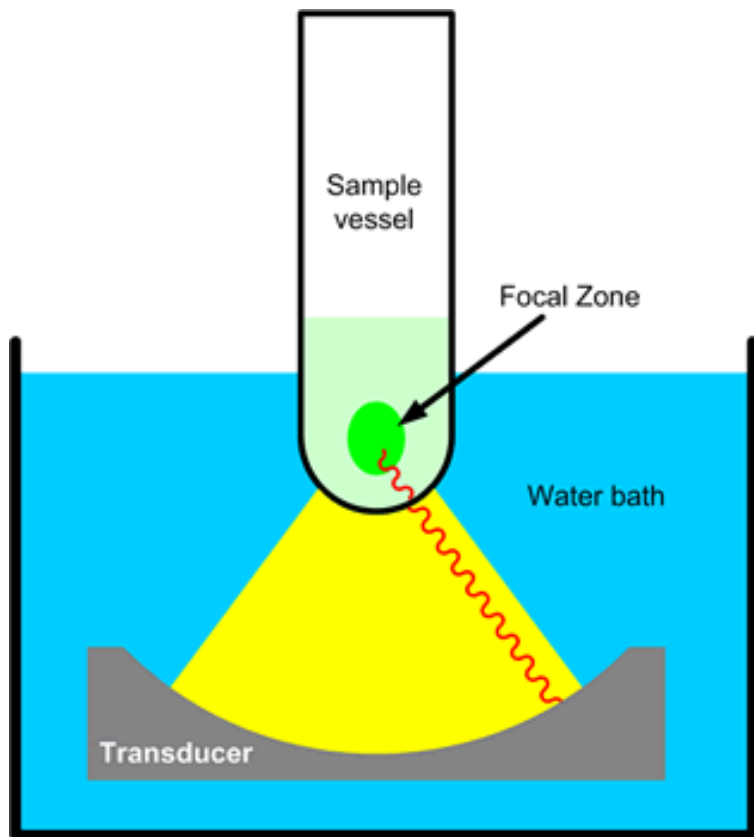


Covaris - 2000

Covaris-2000

- Non-contact
- Isothermal
- Manual & Automated platforms
- Capable of dissolution in open or closed containers
- Ideal for rapid dissolution
- Scalable from large tubes to 1536 well micro-plates
- Highly controllable from high to low power
- Significantly reduces sample thaw time

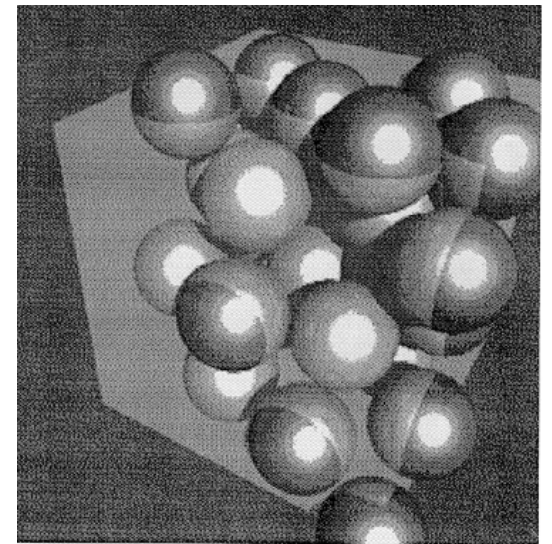
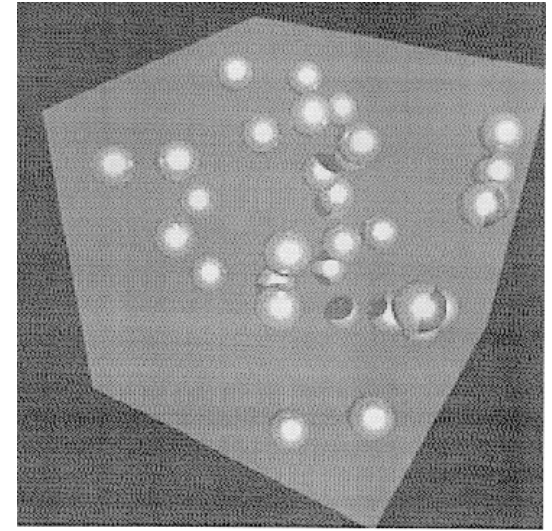
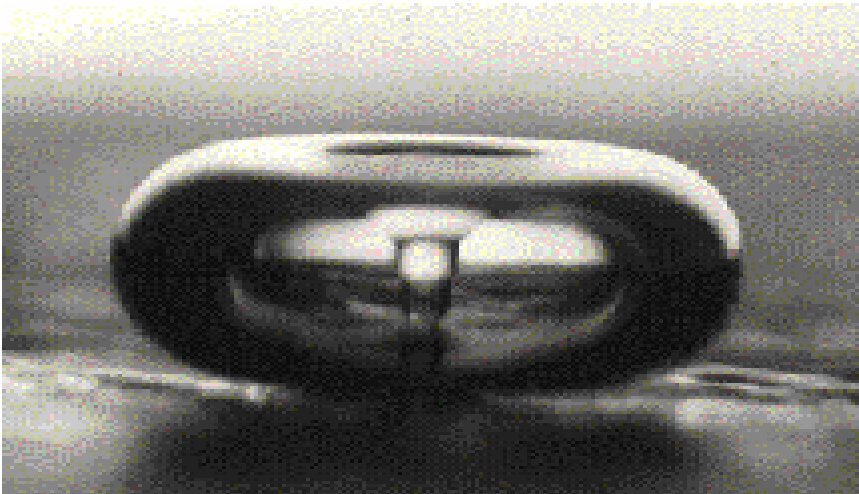
Technology



- Focused, high frequency acoustic waves are emitted from a 400 watt transducer through a water bath and into the open/closed sample vessel.
- 500kHz sound waves are focused into sample creating powerful vortex resulting in rapid dissolution of the sample.
- Extreme vortex dissipates any small heat input into the water bath. As a result, no hot spots are produced and the sample integrity is maintained.

Technology

Dissolution occurs from repeated collapsing of cavitation bubbles. These hollow bubbles expand and contract quickly producing “jets” of water that move at speeds greater than 100m/sec.



Experiments

Treatment time

- How long will it take for most samples to go into solution?

Water uptake

- How much water will uncapped sample absorb over a full bed run time period?

Vortex vs. Sonication vs. Dissolution

- Will a sample react the same way with vortexing, sonicating, and dissolution?

Solubility

- Can poorly soluble samples go into solution with repeated mix cycles?

Degradation

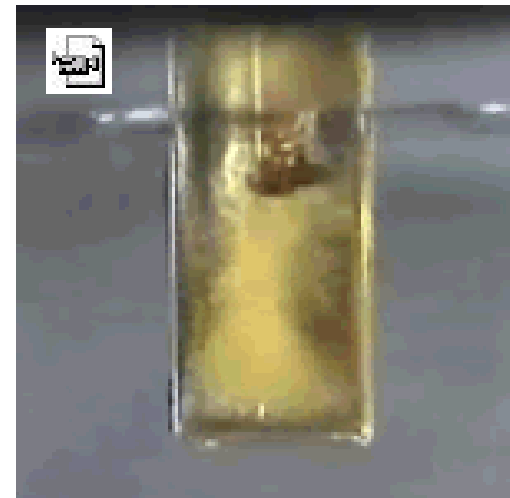
- Will a sample degrade with repeated mix cycles?

Experiments : Treatment Time

400 difficult to solublilise compounds were selected. Of these, 85% were dissolved in **20** seconds.

The remainder were converted to a fine suspension that could be pipetted by a liquid handling robot.

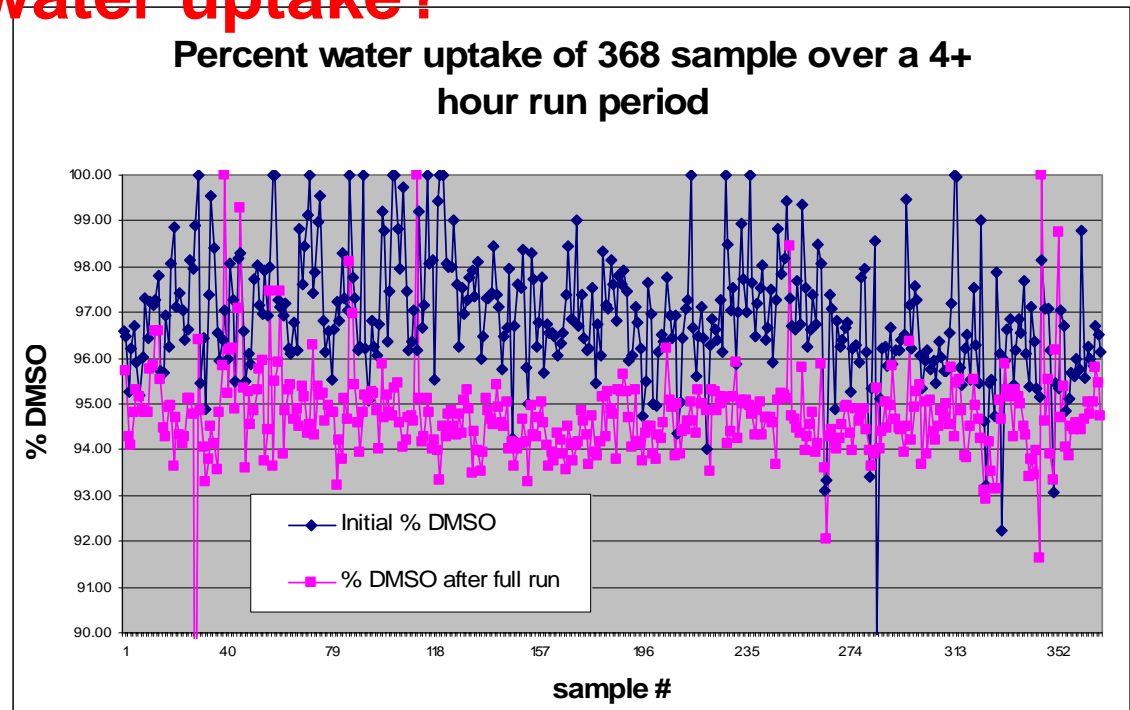
Adaptive Focused Acoustics are capable of dissolution in 20 seconds that would previously take 24 hours sitting at ambient temperature.



Experiments: Water uptake

368 uncapped samples were tested for water uptake in a four hour period during acoustic dissolution. Water uptake was measured using the Echo 555. **how does the echo measure water uptake?**

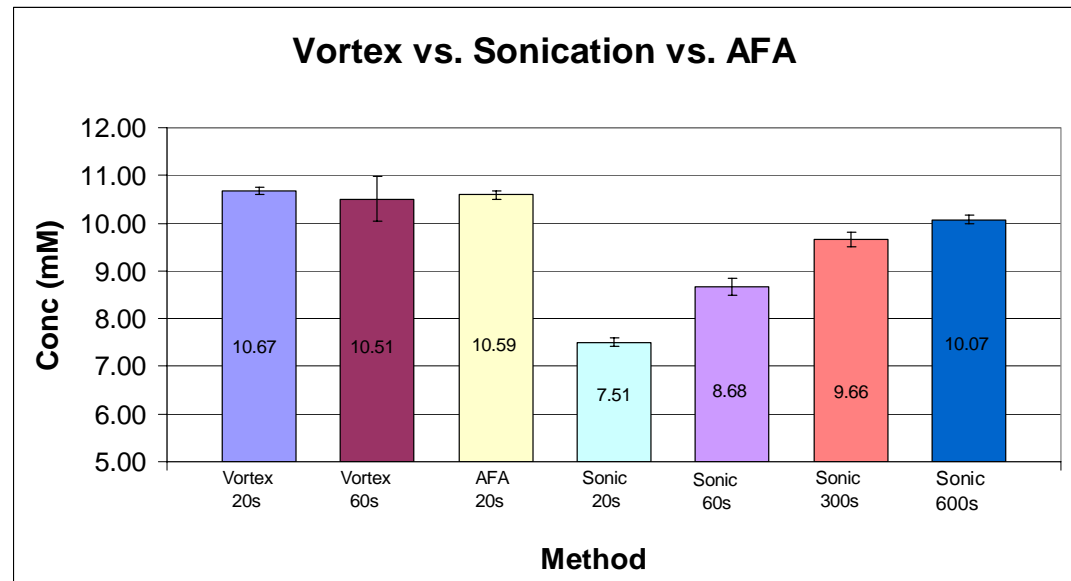
Average water uptake was 2.25%. Control samples confirmed these results.



Experiments: Vortex and Sonication versus Adaptive Focused Acoustics

Albuterol, a difficult to solubilize sample, was dissolved and dissolved using three different methods.

The time mixed ranged from 20-600 seconds and the concentrations were read using the NMR.

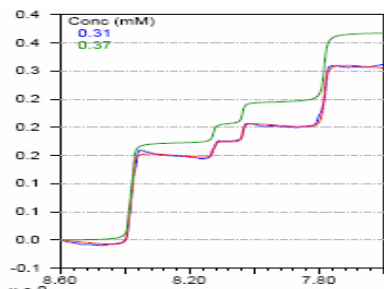


Results indicate Albuterol takes longer to go into solution using sonication bath methods.

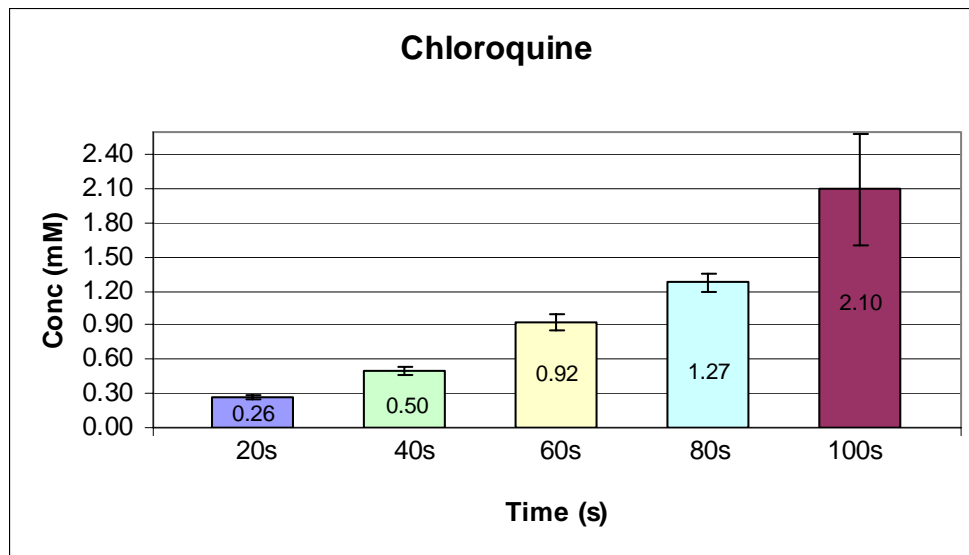
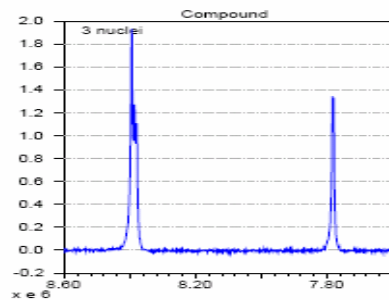
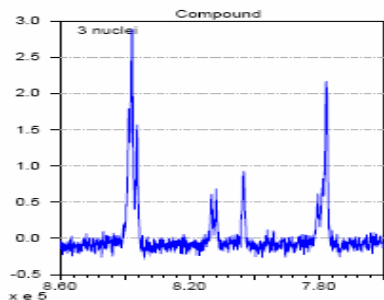
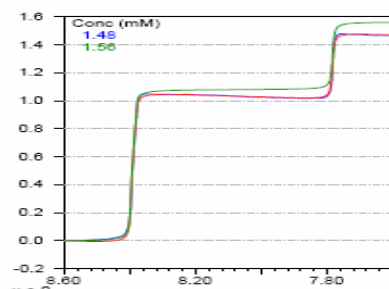
Experiments : Solubility

Chloroquine, an insoluble sample, was acoustically dissolved for a total of 100 seconds. The purpose was to see if over time, an insoluble compound would go into solution.

Well A1(20s)



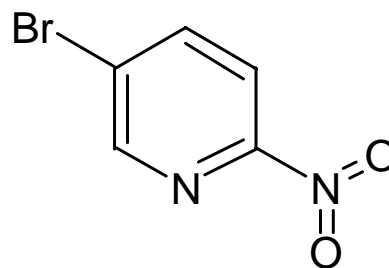
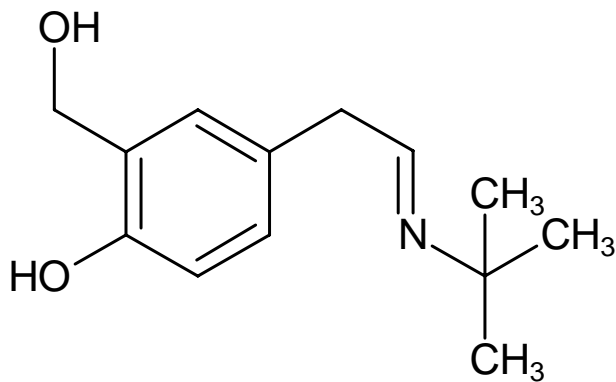
Well B12 (100s)



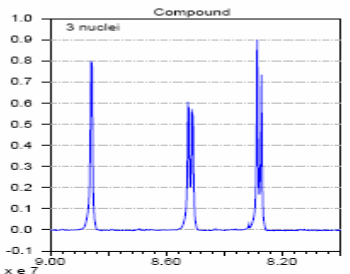
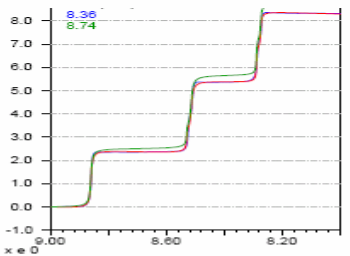
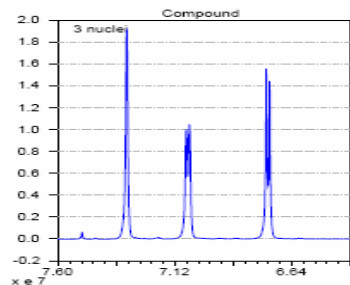
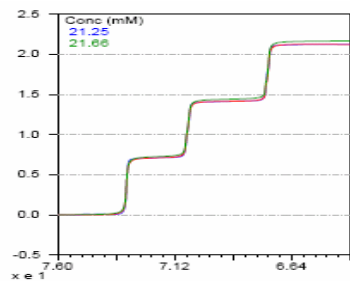
After 100 seconds, Chloroquine went partially into solution as compared to initial 20 second readings.

Experiments: Degradation

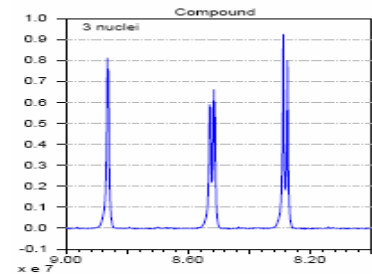
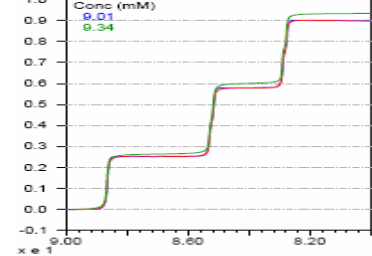
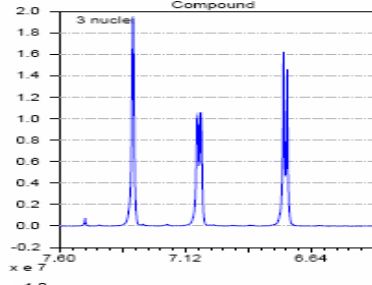
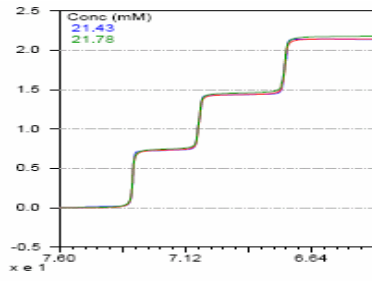
Two samples, Albuterol and 5-Bromo-2-nitropyridine were treated 5 times with 20 second doses. These samples range from very soluble to mostly soluble, reflecting solubility nature of the solid compound store.



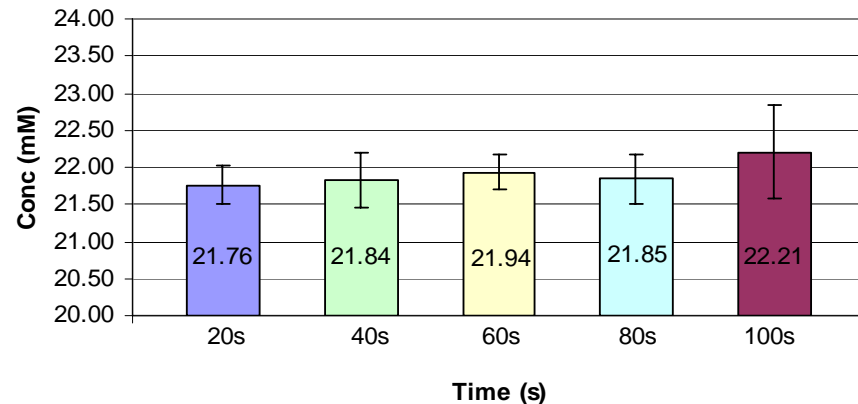
Well A1(20s)



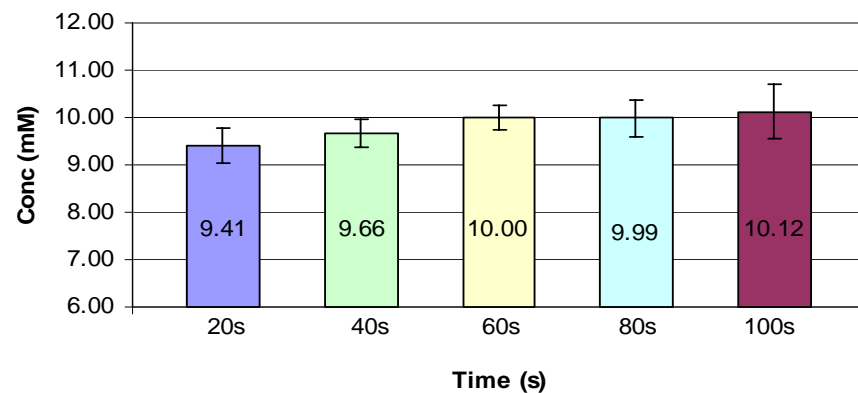
Well B12 (100s)



Albuterol



5-Bromo-2-nitropyridine



NMR results showed no apparent degradation.

Comparison

Sonication	AFA
Gum/Glue dissolution ~ slow	Gum/Glue dissolution ~ 20seconds
Not commonly integrated	Easily integrated & automatable
Vials capped	Vials capped or uncapped
Energy is random and unfocused in the container	Acoustic waves can be focused to a ¼" point
Random convergent sound waves produce significant heat	Residual heat dissipates into water bath - isothermal
Results not reproducible	Results reproducible
Dissolves samples at 20kHz – less effective	Dissolves samples at 500kHz - more effective

Benefits: Process Improvements

Process	Previous Setbacks	AFA improvements
General sample processing	Vortex all samples manually once dissolved and sonicate difficult samples for 15 minutes.	Every sample automatically mixed for 20 seconds each.
High throughput sample processing	Samples dissolved and set aside at room temperature for 24 hours to allow full dissipation.	24 hour turn around period reduced to 4 hours.
2D tube thawing	Tube racks thaw for 20-40 minutes depending on number of tubes.	Thaw time reduced to 5-15 minutes depending on number of tubes.

Next Steps

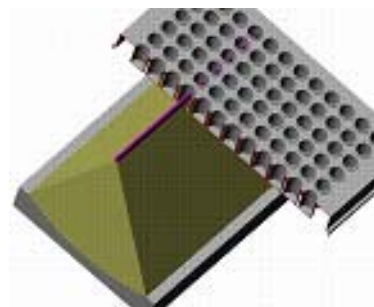
Continued Experimentation

- Potential degradation of sample due to heat from sonication versus isothermal Adaptive Focused Acoustics
- Ability to handle precipitated compounds

Large scale integration into GSK Automated Liquid Stores

- System will replace tube thaw stations that currently take 40 minutes to thaw one tube rack stored at -20° Celsius despite the number of tubes present

Low volume and 1536 well plate mixing



Further GSK Applications

- Low volume plate mixing 1536
- Drug Metabolism and Pharmacokinetics & ADMETox
- High Throughput Chemistry - dissolution
- Compound formulation
- Sono-nucleation
- Membrane prep
- Plasma Extraction
- Cell lysis of E.Coli, Mammalian
- Tissue homogenisation for Metabolic studies
- RNA extraction - Homogenisation of biological tissue
- Tablet disruption for Pharmaceutical Quality Control
- Bead re-suspension – constant suspension

Contributors

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