



Applications of Highly Parallel Sonication in Drug Discovery

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Introduction

Sonication has been in use for decades in several applications relevant to drug discovery. Specifically, it has been used for the following:

- Dissolution of solids
- Dispersion of particulates in liquids
- Sonochemistry and chemical reaction acceleration
- Degassing of solutions

Traditionally, sonication has been performed using ultrasonic baths or probe sonicators. Ultrasonic baths are limited in the amount of energy which can be applied to the samples, and require automation-unfriendly immersion. Probe sonicators are limited in that they can only sonicate one sample at a time. Neither method is well-matched to the parallel, automated techniques which have come to comprise modern screening. A microplate-format sonicator which could sonicate all wells of a plate or tube rack simultaneously would bring the many benefits of sonication to the HTS community.

The SonicMan High-Throughput Sonicator

The SonicMan is a benchtop sonicator which can sonicate all wells of a 96- or 384-well plate or storage rack at one time. It has an easy-to-operate user interface, with control of the instrument via a touch-sensitive panel or external automation controller. A plate or tube rack is placed on a shuttle which extends out of the instrument, then retracts back in for sonication.

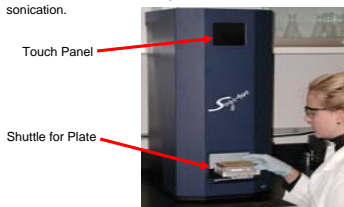


Fig. 1. Front view of SonicMan.

The sonic energy is provided by a sonic horn which is pressed against a pinned lid. The pins carry the sonic energy from the horn into the samples in the microplate or tube rack:

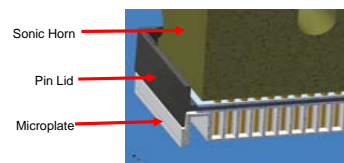


Fig. 2. Diagram showing "sandwich" of sonic horn, pin lid, and microplate which contains samples.

Enhancement of Mixing

Sonication with the SonicMan greatly speeds up mixing in small volumes. This can be easily demonstrated by a simple experiment. 50 μ L of concentrated sucrose colored with red dye was placed in 1.5-mL Micronics tubes, and covered with 500 μ L of DI water. Absent any agitation, mixing is not complete even after eight hours. By contrast, the SonicMan achieves complete mixing in 20-40 seconds:

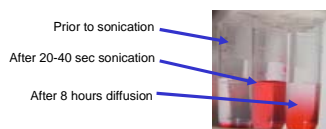


Fig. 3. 1.5 mL Micronics tubes containing dyed sucrose solution and water before sonication, after sonication, and after 8 hours diffusion.

The evenness of mixing can be visualized in a 96-well plate using a two-component system similar to that described above, but using a pH-sensitive dye (bromothymol blue, BTB) and selecting the starting pH and buffering capacity of the two components. The dye starts out in the blue form, but quantitatively converts to the yellow form on mixing. After 40 sec, all but one wells are completely mixed, and after 50 sec, all wells are mixed:

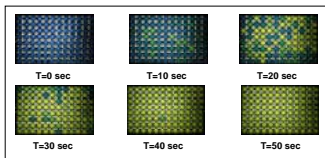


Fig. 4. 96-well tube rack viewed from top. Each tube initially contains acidic sucrose solution in bottom, and BTB in alkaline buffer above. Color change to yellow indicates complete mixing. Mixing is complete after 50 sec.

Figure 5 below shows the time profile of the absorbance at 615 nm (blue dye form) for a 384-well plate with 70 μ L per well. Full mixing in all wells is achieved in 40 sec. [Dye color transition in this experiment is yellow to blue].

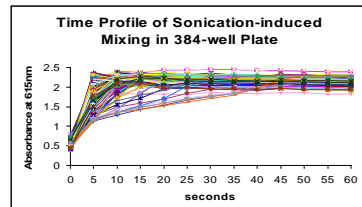


Fig. 5. Time profile of sonication in 384-well plate. Maximum, unchanging absorbance of BTB measured at 615 nm indicates complete mixing after 40 sec of sonication.

Enhancing Solubilization

Screening compounds stored in DMSO may be anywhere from completely dissolved to completely precipitated. Screening such samples without considering the solubilization can result in missed hits, unnecessarily sparse SAR data, and incorrectly high estimates of IC₅₀. Sonication prior to liquid handling or screening insures that compounds are back in solution to the limits of their thermodynamic solubility.

Figure 6 below shows results from five selected 96-well storage racks (80 cmpd/plate) out of a large number which were being prepared for screening. Each sample tube in each rack was individually inspected for precipitation. The racks were then sonicated using SonicMan, and re-inspected. The graph shows the percentage of precipitated samples which were clarified by sonication. The improvement in solubilization ranges from around 25% up to 70%.

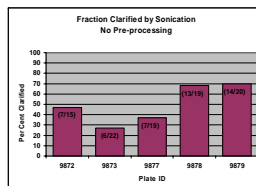


Fig. 6. Enhancement of solubilization in five selected 96-compound plates during preparation for a screen. Second value in parentheses is the number of initially precipitated samples. First value is the number of those precipitated samples which were put back into solution by sonication.

When DMSO solutions of compounds are pipetted into aqueous buffer for screening, compounds can precipitate due to the change of solvent environment. Typically, such samples need to be shaken overnight to accomplish mixing and solubilization of compounds. Sonication can shorten that process to a matter of seconds.

In the experiment described below, three different methods of mixing are compared for 22 biologically active compounds of varying aqueous solubility. The amount of compound in solution in each case was measured using the NanoStream Velocose system. Results for the three methods are comparable for each compound, showing that sonication can achieve solubilization within seconds to the solubility limit of the compound.

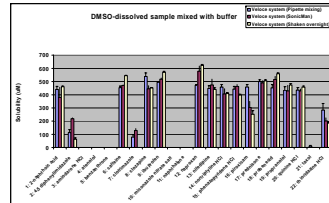


Fig. 7. Comparison of three solubilizing methods for 22 compounds diluted from DMSO stocks into aqueous solution.

Enhancement of Bioassay Results

If an active compound is not in solution when assayed, it is highly unlikely that it will show up as a hit. Sonication all samples prior to liquid handling in screening ensures that the compounds are solubilized to the limit of their solubility. Re-solubilization of precipitated compounds will facilitate a positive assay result for active compounds.

Figure 8 shows assay results for a compound storage plate which had been through several freeze-thaw cycles. Without sonication, the plate showed three hits. When the plate was sonicated prior to performing the assay, several more hits were seen:

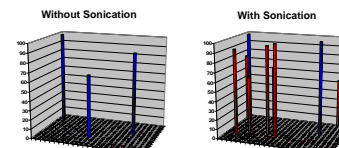


Fig. 8. Screening results for a 384-well plate-based biochemical assay, both without sonication and with sonication. Vertical axis is per cent activity.

Sonication prior to a concentration-response assay can have a dramatic effect on the measured IC₅₀. Figure 9 below shows the results for thirteen randomly selected compounds which were assayed with and without sonication of the compound prior to aliquoting for the concentration-response experiment. While more than half of the compounds showed no or minor change in the IC₅₀ when sonicated prior to the assay, five of the thirteen compounds (38%) showed roughly two orders of magnitude or greater decrease in measured IC₅₀ as a result of sonication.

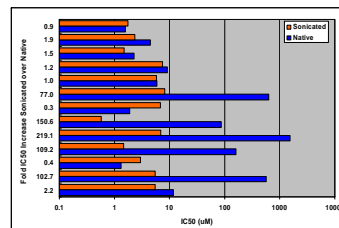


Fig. 9. IC₅₀ results for thirteen random compounds which showed screening activity, with and without sonication prior to assay. Five of the thirteen showed approximately two orders of magnitude improvement in measured IC₅₀ with sonication.

Biology Applications

Sonication has multiple applications in biology, among which are the following:

- Cell lysis
- Loading of macromolecules into cells
- Inactivation of microorganisms
- Tissue disruption and homogenization

Figure 10 below shows the effect of increasing sonication on suspended mammalian and bacterial cells as measured by the protein released into medium. In both cases, the amount of protein increases up to a limit which is consistent with the amount of protein released by a commercial protein release agent. Maximal protein release is accomplished in less than 30 sec.

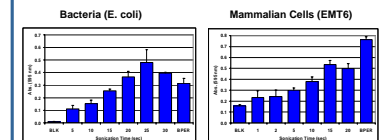


Fig. 10. Sonication-induced protein release from bacterial and mammalian cells, measured with the Bradford reagent. BPER is a commercial bacterial protein releasing agent from Pierce.

Conclusions

The SonicMan brings the many known beneficial applications of sonication to the high throughput arena. Applications such as compound dissolution, mixing, and cell lysis can be performed in a highly parallel, automation-friendly manner. Screening results will be enhanced proportional to the aggregate degree of compound precipitation in a given library. Any other applications of sonication which would benefit from high throughput are in principle approachable with the SonicMan.

Acknowledgements

The authors graciously acknowledge the contributions of several collaborators in the pharmaceutical industry:

- Pfizer: Chris Lipinski, Michele Kelly
- Aventis: Tina Garyantes, Julie Lee, Tony Lozada, Yong Yan
- Nanostream: Steve Hobbs, Paren Patel, Courtney Coyne
- Chiron: Tim Dawes
- Matrical: Doug Pooler, Hans Harzl, Bernie Polikowski

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