High-throughput, low-volume determination of thermodynamic solubility using sonication and micro parallel liquid chromatography

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Abstract

Determining the thermodynamic solubility of a drug compound under various conditions and in different formulations is a very time- and compound- consuming process. Typically, a compound, in excess of its solubility, is placed in a shaker flask and the test buffer is added. The flask is then shaken for 24-48 hours until equilibrium is reached. The concentration of the dissolved sample is then determined by HPLC after separation of the solid phase by filtration or centrifugation. This method to determine the thermodynamic solubility requires a significant amount of time (typically up to 1 week), gram quantities of materials, and numerous HPLC systems to analyze all of the samples.

By combining a highly parallel sonication system, SonicMan, with a micro parallel liquid chromatography (µPLC) system provided by Nanostream, the amount of material needed for analysis, as well as the time needed to achieve the results can be dramatically reduced. Here we present data on the measurement of thermodynamic solubility of several compounds under a variety of conditions. This poster compares the measured solubilities of these compounds as determined by the classical shaker flask method with those obtained by combining plate-based sonication in the SonicMan with analysis using a system for parallel liquid chromatography provided by Nanostream.

Introduction

The Veloce µPLC system (Figure 1) was developed to provide increased sample analysis capacity. The system uses established HPLC techniques and integrates with conventional laboratory workflow to increase time and cost efficiency. At the heart of the Veloce system is the 24-column Brio cartridge, which enables 24 samples to be analyzed in parallel for increased analytical throughput. The utility of this system has been demonstrated for high-throughput analytical applications, including compound purity assessment and thermodynamic solubility measurements.[1,2]

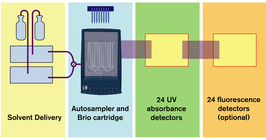

Figure 1: Veloce µPLC system schematic

The SonicMan uses sonic energy to drive compounds into solution. Relying on a pinned lid placed on a 96 or 384-well micro plate, SonicMan technology employs a sonic horn to transfer sonic energy through the lid into the sample to resuspend compounds in solution without cross contamination. Applications include: resuspending stored compounds for HTS; resolubilizing compounds in synthesis; shearing genomic data into small fragments for PCR amplification; extracting target proteins, RNA/DNA, and enzymes via cell lysis and membrane disruption; mixing low volume assays; and transfecting eukaryotic cells.

The combination of the Veloce µPLC system and the SonicMan offer the following benefits compared to conventional methods such as shake-flasks with HPLC detection:

- Dramatically reduce volumes. Only 50 µL or less is needed to perform measurements.
- Save time while still providing reliable results. Test materials can be mixed in seconds and analyzed in a high throughput platform using proven LC methods.
- Simplify workflow. Both systems are designed to work with standard microplates. Reagents do not need to be transferred from flask to vial.

Data is presented here to demonstrate the combined use of the Veloce micro parallel liquid chromatography system and SonicMan ultrasonic plate mixer for high-throughput solubility measurements.

Materials and Methods

Equipment

- Veloce µPLC system (Figure 2) (Nanostream, Pasadena, CA)
- Brio cartridge with 24 parallel LC columns [80 x 0.5 mm] packed with C18 (Figure 3)
- SonicMan ultrasonic mixer for 96- and 384-well micro plates. (Figure 4) (Matrical, Spokane, WA)
- Filter plates (Millipore, part # MSSL BPC 10, Billerica, MA)

Figure 2: Veloce µPLC system with binary gradient module, 24 UV absorbance detectors, and 8-head autosampler for use with 384-well plates

Figure 3: Brio cartridge with 24 microfluidic LC columns [80 x 0.5 mm]

Methods

Method for determination of compound solubility.

1. Materials were mixed. Materials and mixing methods are described with results.
2. Resulting samples were filtered through a 96-well micro plate filter unless noted otherwise.
3. Air bubbles were removed from filtrate by centrifuging for 3 minutes at 3200 times g.
4. Samples were analyzed on the Veloce µPLC system and standard curves were generated.

Analytical Conditions

- Injection volume: 0.5 µL.
- Flow rate: 12.5 µL/min (for each column).
- Mobile phase A: 95:5 water:acetonitrile w/0.1% formic acid; B: Acetonitrile w/0.085% formic acid.
- Gradient method: t = 0 min, 10% B; t = 0 to 1:00 min, 10% B; t = 1:00 to 5:30 min, ramp to 100% B; t = 5:30 to 7:00 min, 100% B; t = 7:00 to 10:00 min, 10% B.
- UV absorbance detection, λ = 214 nm.

Figure 4: SonicMan ultrasonic microplate mixer

Results and Discussion

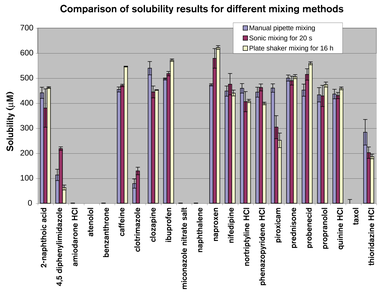

Figure 4. Comparison of solubility results for different mixing methods. 10 µL of 10 mM compound in DMSO was mixed with 190 µL of buffer (pH 7.4). Thus maximum solubility values are 500 µM. (A) Manual pipette mixing, (B) SonicMan for 20 seconds and (C) Plate shaker for 16 hours.

- All methods produced similar results for all compounds, with the exception of low solubility values for overnight shaking of clotrimazole.
- The SonicMan allowed rapid, automated mixing. Mixing with the SonicMan can be completed in 20 seconds whereas 1.5 hours is recommended for a plate shaker.[3]
- Use of µPLC allowed for high throughput analysis compared to HPLC. To use the procedure described here as an example, 3 samples and 4 standards were run for each compound. Analysis of 24 compounds (168 separations) requires 2.0 hours on the Veloce system and 30.8 hours on a traditional HPLC.
- Atenolol co-eluted with DMSO and was not detected in any of the experiments presented here.
- The entire method was highly parallel. Microplates and multichannel pipettes were used throughout the process, thus avoiding tedious handling of individual samples.

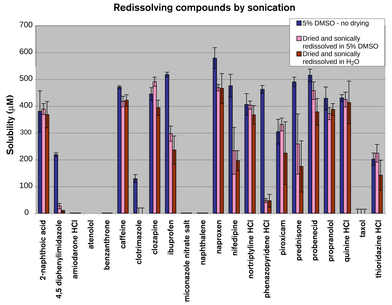

Figure 5. Solubility determined for samples redissolved in DMSO and aqueous buffer. 2.5 µL of each 10 mM compound were added to individual wells in a 384 well plates. The DMSO was then vacuum evaporated and 50 µL of buffer was added to each well. (A) Solubility in 5% DMSO (B) Solubility determined after drying and sonic reconstitution into 5% DMSO (C) Solubility determined after drying and sonic reconstitution in aqueous buffer.

- Solubility can be determined in volumes as low as 50 µL.
- Aqueous solubility can be determined from samples originating in 5% DMSO solutions.
- As expected, solubility is near or lower in purely aqueous solution than in 5% DMSO.
- The SonicMan can be used for rapid reconstitution of solid compounds.
- Most compounds are almost fully reconstituted to their previous concentration.

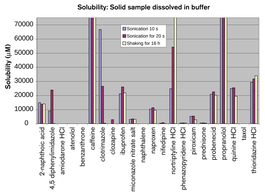

Figure 6. Solubility determined from dissolution of compounds in solid form. To individual wells of a 96-well plate, 10-20 mg of compound and 200 µL of pH 7.4 buffer were added. Dissolution was performed by two means (A) by sonication using the SonicMan and (B) by plate shaker.

- The SonicMan can be used for rapid dissolution of solid compounds.
- Results using sonication were very similar to a plate shaker operated for 16 hours. Two exceptions are compounds 4,5 diphenylimidazole and clotrimazole. The two anomalies could be due to limited dissolution by the plate shaker.
- Solubility values for 20 seconds of sonication were comparable to values for 10 seconds. There was no apparent pattern in differences between values for sonication and plate shaker.

Conclusion

- The combined use of SonicMan, microplates and µPLC allows for rapid determination of compound solubility.
- Solubility measurements can be determined from very small (50 – 200 µL) sample volumes, conserving costly or limited compounds.
- The entire process described here is highly parallel. Sample handling is performed using 96 or 384-well microplates thus avoiding tedious handling of individual vials and single-channel pipetting.
- Samples can be reconstituted to their thermodynamic solubility in less than 20 seconds.

References

1. Lemmo et al. "Micro parallel liquid chromatography: enabling technology for discovery analytical chemistry." *Assay and Drug Development Technologies*, Vol. 2, Number 4. In press.
2. Hobbs et al. "Determination of thermodynamic solubility and permeability using filtration-based 96-well plates and 24-column micro parallel liquid chromatography system." SBS 2004, Orlando, Florida.
3. Millipore Protocol Note # EC2445EN00.

About MatriCal

MatriCal is a leading developer, manufacturer, and supplier of laboratory instrumentation and consumables to the life science research market.

The SonicMan high throughput sonication system paves the way for improved drug dsicovery. Applications include: resuspending stored compounds in HTS; resolubilizing compounds in synthesis; shearing genomic data into small fragments for PCR amplification; extracting target proteins, RNA/DNA, and enzymes via cell lysis and membrane disruption; mixing low volume assays; driving sonochemical reactions; and transfecting eukaryotic cells.

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