A New Aqueous Solubility Screen: High-throughput Sonication

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Abstract:

Poor compound solubility and inefficient methods to drive compounds to their thermodynamic solubility can decrease productivity in drug discovery and development[1]. Although the concept of high-throughput screening has been integrated into the pharmaceutical drug discovery process it is not as of yet useful in compound solubility as the dissolution of drug compounds to saturation is still reliant on the inherently low-throughput[2] and time consuming shake-flask method[3]. The SonicMan (MatriCal, Inc., Spokane, WA) introduces a high-throughput method for compound dissolution with a microplate format sonicator. In this study the SonicMan is comparatively evaluated against the conventional shake-flask method for their respective compound dissolution properties. Results indicate that the SonicMan is a viable option for determining aqueous drug solubility in this high-throughput era of drug research.

Introduction:

The discovery and development of new drug compounds in the pharmaceutical industry is both time and monetarily intensive due to the high attrition rates of potential compound candidates[4]. Therefore screening strategies that are able to rapidly and accurately screen out unfavorable compounds based on their ADME (absorption distribution, metabolism, and excretion) profiles and more fundamentally their physiochemical properties could lower the resources used on these 'unworthy' compounds. One response to the 'high attrition rate' seen by the drug discovery industry is the relatively new trend to evaluate compound solubility early on in drug development parallel to efficacy optimization. Countless resources have been wasted on lead compounds that had promising *in vitro* potency yet failed subsequently due to unfavorable solubility properties in later physiochemical tests. Solubility in ADME profiling falls under the 'absorption' category and is important as the aqueous solubility predicts the maximum amount of the drug compound available for intestinal absorption[5]. Over-valuing or under-valuing of a compound's solubility might lead to inappropriate development strategies for that compound or lead to erroneous results like 'false negatives' in a bioassay screening test. Measuring solubility in a swift and accurate manner is problematic in drug development especially when attempting to meet this industry's high-throughput requirements. The dissolution process of a compound equilibrating in a buffer is a slow time consuming process in drug discovery which has traditionally been accomplished by the shake-flask method which is fundamentally low-throughput and labor/time intensive. Other methods which try to estimate an 'apparent solubility' based kinetic profiles or DMSO corrections are irreproducible and prone to errors. Sonication has been shown to dissolve compounds to their thermodynamic limit[6] rapidly but has not been of use for dissolution in drug discovery due to the lack of a high-throughput sonicator capable of meeting the depends of modern drug discovery screening. MatriCal has designed a microplate sonicator capable of sonication in 96, 384, and 1536 well formats which is able to fill this niche in drug discovery research.

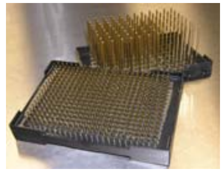
Table 1: Compound Information

Compound Name	Supplier	Cat #	CAS #	MW	MP	pKa acid	pKa base	Type	logS*	Ref
19-Norethindrone acetate	Sigma-Aldrich	N6127-100MG	51-98-9	340.47	206			Neutral	-4.79	7
Acetaminophen	Sigma-Aldrich	A5000-100G	103-90-2	151.17	167	9.86	1.72	Neutral	-1.074	8
Albendazole	ICN	193912	54965-21-8	265.30	209	11.39	5.62	Neutral	-4.42	9
Ampicillin	Sigma-Aldrich	271861-5G	69-53-4	349.41	203-204 (d)	2.44	6.76	Zwitterion	-1.539	8
Aspirin	Sigma-Aldrich	239631	50-78-2	180.16	135	3.48		Acid	-1.73	8
Baclofen	Sigma-Aldrich	B5399	1134-47-0	213.70	206-208	4.04	10.32	Zwitterion	-1.696	8
Caffeine	Sigma-Aldrich	C0750-100G	58-08-2	194.19	232		0.73	Neutral	-.951	8
Carbendazim	Sigma-Aldrich	378674-100G	10605-21-7	191.19	300	11.97	6.09	Base	-4.50	10
Chloroquine diphosphate	Sigma-Aldrich	415480-25G	50-63-5	515.87	213		10.47	Base	-.71	9
(+) Dehydroisoandrosterone	Sigma-Aldrich	125784-2.5G	53-43-0		152-153	15.02		Neutral	-4.01	7
Dexamethasone	Sigma-Aldrich	D1756-100MG	50-02-2	392.47	262-264			Neutral	-3.61	7
Diclofenac sodium salt	Sigma-Aldrich	D6899	15307-79-6	318.1	157	4.0		Acid	-5.097	8
EDTA disodium salt	Sigma-Aldrich	106313-5G	6381-92-6	372.24	252 (d)	1.78	11.24	Acid	-.018	8
Estrone	Sigma-Aldrich	E9750-500MG	53-16-7	270.37	260			Neutral	-5.53	7
Fenbendazole	Sigma-Aldrich	F5396-5G	43210-67-9	299.40	298	11.08	5.47	Neutral		
Griseofulvin	Sigma-Aldrich	G4753-5G	126-07-8	352.77	216			Neutral	-4.60	7
Haloperidol	ICN	153696	52-86-8	375.90	151.5	13.85	8.25	Base	-4.43	7
Ibuprofen	Sigma-Aldrich	I4883	15687-27-1	206.28	76	4.4		Acid	-3.76	7
Loperamide HCl	ICN	153676	34552-83-5	513.50	222-223	13.85	8.05	Base	.12	
Mebendazole	Sigma-Aldrich	M2523-25G	31431-39-7	295.30	288.5	10.65	5.02	Neutral	-4.48	7
Mianserin HCl	Sigma-Aldrich	M2525-100G	21535-47-7	300.8			8.25	Base		
Naproxen	Sigma-Aldrich	284785	22204-53-1	230.26	153	4.15		Acid	-4.155	8
Nicardipine HCl	Sigma-Aldrich	N7510-1G	54527-84-3	516.00	168		7.29	Base		
Pimozide	Sigma-Aldrich	P1793-500MG	2062-78-4	461.60	214-218	12.04	9.42	Base	-5.20	11
Progesterone	Sigma-Aldrich	P8783-1G	57-83-0	314.47	128			Neutral	-4.42	7
(+/-) Propranolol HCl	Sigma-Aldrich	P0884-1G	318-98-9	295.81	163-165	13.84	9.14	Base		
Reserpine	Sigma-Aldrich	R0875-1G	50-55-5	608.69	265	18.11	7.25	Base	-4.42	9
Salbutamol sulfate	Avocado	A18544	51022-70-9	576.27		9.99	9.22	Base		
Tetracycline	Sigma-Aldrich	T3258-5G	60-54-8	444.44	172	4.50	11.02	Zwitterion	-3.28	12
(+/-) Thalidomide	Sigma-Aldrich	T144-100MG	50-35-1	258.20	239-241	10.70	-2.55	Neutral	-3.699	8

The SonicMan™

The SonicMan™ is a high throughput multi-probe sonication instrument developed by MatriCal, Inc. configurable with 96, 384, and 1536 well formats (Fig 1A). The Instrument is operated with a user-friendly touch screen interface. The SonicMan uses disposable gasketed pin lids (Fig 1B) to transfer sonic energy to each individual well and ensure no well to well cross contamination. Plates are placed on a extendable/retractable shuttle. The sonicater allows for power outputs between 100 and 1150 watts (12 watts/pin for 96 well formats and 3 watts/pin for 384 well formats at 100% power) and configurable time intervals from .1 to 20 seconds.

- High-throughput sonicator compatible with 96, 384, and 1536 plate formats.
- Easy, rapid, and efficient dissolution of a compound library.
- Efficient re-dissolution of stock DMSO compound libraries.



96 & 384 Pinned Lids

Figure 1A:

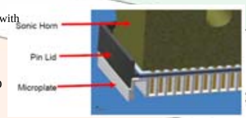


Figure 1b: **Diagram showing "sandwich" of sonic horn, pin lid, and microplate which contains samples.**

Materials & Methods:

All compounds used in this study were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO) or ICN (MP Biomed, Irving, CA). All compounds were obtained in dry powder form and were examined at or near room temperature. The compounds used were chosen to represent those found in a typical screening library.

Mini-Shake-Flask Method:

Compounds were weighed into 1.4 mL polyethylene storage tubes commonly used for compound storage in the drug screening industry (Matrix, Hudson, NH). The amount of each compound was sufficient to ensure saturation when mixed with 750*u*L of Phosphate Buffered Saline (PBS), pH 7.2, in 1.4mL tubes at room temperature. These tubes were placed in their corresponding 96-well-format rack on a plate shaker (IKA MTS4) at a setting of 1000min^{-1}. From each tube 100 *u*L aliquots of the suspension were withdrawn as soon as the shaker was stopped at times 0, 4 hours, 8 hours, 24 hours, and 48 hours of shaking. Each 100 *u*L aliquot was then centrifuged using a Galaxy 7 (VWR, West Chester, PA) at 6500 rpm for approximately 5 minutes to separate the supernatant from any excess solid. Aliquots of 50uL were then taken from the supernatant and diluted an appropriate amount for spectroscopic analysis. Carbendazim, Dehydroisoandrosterone, Haloperidol, and Loparamide HCl were also analyzed using the same protocol but in buffers PBS, pH 2.25, Citrate Buffer, pH 4.85, and PBS, pH 12.10 to assess the effect of varying pH on solubility.

Figure 2: The SonicMan™

Sonication by SonicMan:

Compounds were prepared as in the shake-flask Method and the 1.4 mL tubes were placed in a 96-well-format Matrix tube rack. The solutions were then sonicated for 5, 10, and 20 seconds at 100% power using a pin lid matched to the tube depth (MPL096-P42, MatriCal, Spokane, WA). Immediately after each sonication, 100*u*L aliquots were withdrawn and centrifuged as in the shake-flask method to pellet any undissolved compound. Aliquots of 50*u*L were then withdrawn from the supernatant and diluted an appropriate amount for spectroscopic analysis. The UV absorption of 7 compounds was also determined 1 hour, 1 day, and 1 week subsequent to 20 seconds of sonication to determine if there was any sonication induced supersaturation.

Spectroscopic Analysis:

All dissolved compounds were analyzed on a Shimadzu UV-1601 UV-Visible spectrometer at a wavelength which had been previously determined for each compound to a significant absorbance. This method allowed direct comparison of the different treatments of the same compound without the requirement for absolute determination of concentration in solution.

Results & Discussion:

30 known biologically active compounds (See Table 1) were chosen with a range of physical properties representative of that which would be found in a pharmaceutical company's screening compound library. Compounds of varying aqueous solubility, pKa values, and LogP values were tested for their thermodynamic and kinetic solubility by dissolving them both by shaking and by sonication with the SonicMan. The traditional method for measuring the solubility of drug compounds is the shake-flask method which often involves shaking the compound for 24 to 48 hours to reach thermodynamic equilibrium. In general the data produced in this experiment shows that a compound that takes 24 to 48 hours to reach its thermodynamic equilibrium solubility value by shaking can be kinetically driven to that same solubility value in seconds by sonication. As shown in figures 3-4, 16 out of the 21 compounds that had appreciable solubility were dissolved more in 20 seconds of sonication than in 48 hours of shaking, and the remaining 5 compounds showed good agreement between the shake method and the sonication method.

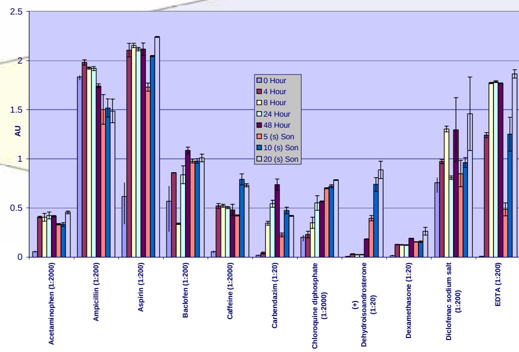


Figure 3: Comparison of the dissolution of compounds achieved by a variation of the shake-flask method (Bars 1-5) vs sonication with the SonicMan (Bars 6-8). Parenthesis is the dilution factor used. All compounds absorption recorded at a predetermined absorption maximum. 8 out the 11 compounds showed more dissolution with 20 seconds of sonication than 48 hours of shaking while the remaining 3 were in good agreement.

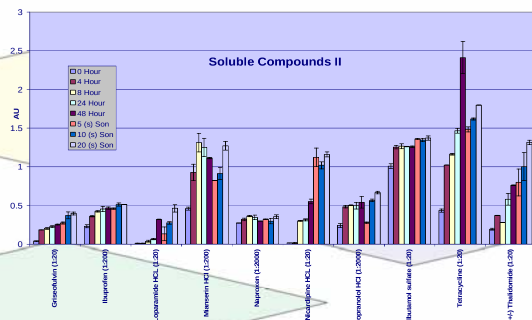


Figure 4: Comparison of the dissolution of compounds achieved by a variation of the shake-flask method (Bars 1-5) vs sonication with the SonicMan (Bars 6-8). Parenthesis is the dilution factor used. All compounds absorption recorded at a predetermined absorption maximum. 8 out the 10 compounds showed more dissolution with 20 seconds of sonication than 48 hours of shaking while the remaining 2 were in good agreement.

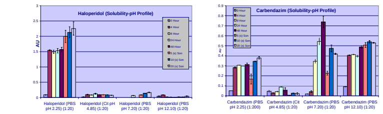


Figure 5: Solubility-pH Profile

The data indicates that varying the pH of the buffer has no affect on the SonicMan's compound dissolution capabilities relative to the shake-flask method. Solubility increases below pH of 2 for Haloperidol are due to the increased solubility of the hydrochloride salt relative to the free base. The hydrochloride salt crystal form of Carbendazim ionizes producing acidic and basic moieties and a higher aqueous solubility.

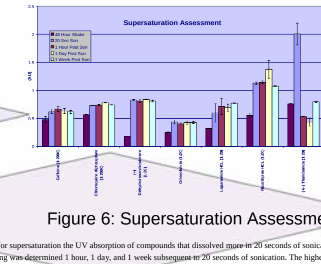


Figure 6: Supersaturation Assessment

To test for supersaturation the UV absorption of compounds that dissolved more in 20 seconds of sonication than in 48 hours of shaking was determined 1 hour, 1 day, and 1 week subsequent to 20 seconds of sonication. The higher solubility's seen by sonication are most likely due to agitation produced by sonication giving a 'de-aggregating affect'. The drop off seen in Thalomide's solubility is most likely due to a sonication induced change in form from crystalline form to amorphous form which then re-crystallizes to its thermodynamically favored crystalline form.

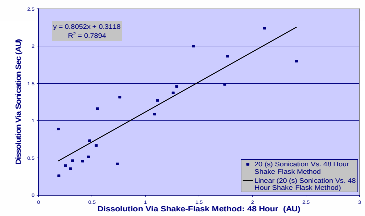


Figure 7: The solubility data obtained with 20 seconds of sonication by the SonicMan and with 48 hours of shaking with the shake-flask method is displayed in the figure. As shown, the SonicMan data is in good agreement with the shake-flask method data in many cases. However, we believe that the shake flask method underestimates the thermodynamic equilibrium solubility for many of the compounds tested. Each point in the figure represents an absorption value (20 sec Son. Vs. 48 Hr shake-flask) from 1 of the 21 compounds displayed in figures 3-4 and 9 insoluble compounds (data not shown).

Conclusion:

- The SonicMan can provide the same results as the low-throughput, labor, and time intensive shake-flask method in seconds.
- The SonicMan offers a high-throughput method in 96 and 384 well formats compatible with standard laboratory robotics.
- SonicMan offers an easy method to re-dissolve compound libraries that have precipitated.
- Reproducible results and no error associated with DMSO contamination as in common filter plate techniques.
- Usable in a variety of solvents and pH ranges.

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