

## **Abstract:**

Poor compound solubility and inefficient methods to drive compounds to their thermodynamic equilibrium can decrease productivity in drug discovery and development<sup>(1)</sup>. 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<sup>(2)</sup> and time consuming shake-flask method<sup>(3)</sup>. 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 drug solubility in this high-throughput era of drug research.

## **Introduction:**

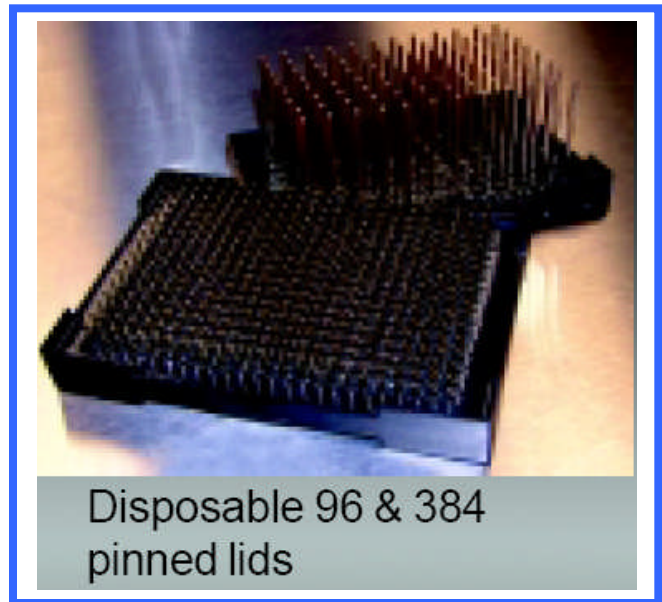
The discovery and development of new drug compounds in the pharmaceutical industry is both time and monetarily intensive due to the high attrition rates of potential compound candidates<sup>(4)</sup>. Therefore screening strategies that are able to rapidly and accurately screen out unfavorable compounds 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. Countless resources have been wasted on lead compounds that showed promise only to show unfavorable solubility properties in 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<sup>(5)</sup>. 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 a problem in drug development especially when attempting to meet this industry's high-throughput needs. The dissolution process of a compound equilibrating with its 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<sup>(6)</sup> rapidly but have not been of use in dissolution in drug discovery due to the lack of a high-throughput sonicator capable of meeting the demands 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.

# The SonicMan™

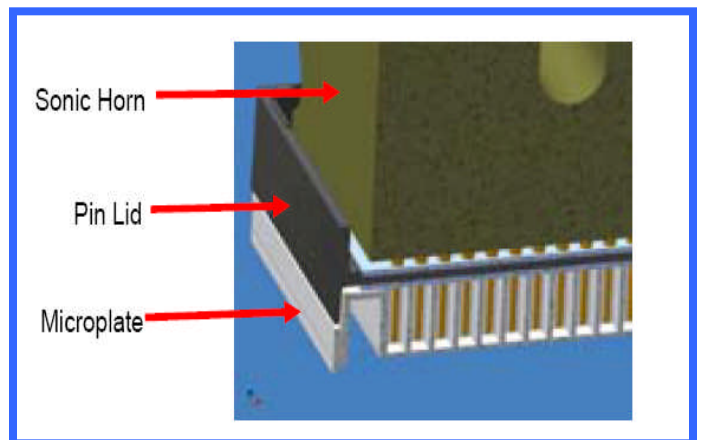
## Figure 1A - 1B:

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 an 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.



**Figure 1A:**  
Disposable 96 and 384 pinned



**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

### Figure 2:

at or near room temperature. The compounds used were chosen to represent those found in a 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  $\mu$ L of Phosphate Buffered Saline (PBS), pH 7.2, in 1.4 mL 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 1000  $\text{min}^{-1}$ . From each tube 100  $\mu$ 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  $\mu$ 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 50  $\mu$ L 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 with the plate shuttle

### 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 pinned lid matched to the tube depth (MPL096-P42, MatriCal, Spokane, WA). Immediately after each sonication, 100  $\mu$ L aliquots were withdrawn and centrifuged as in the shake-flask method to pellet any un-dissolved compound. Aliquots of 50  $\mu$ L were then withdrawn from the supernatant and diluted an appropriate amount for spectroscopic analysis.

## **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 3-5, 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. As shown in figure 6, varying the pH has no effect on the SonicMan's dissolution capabilities relative to the shake-flask method.

## **Conclusions:**

- 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.

## References:

- 1.) Avdeef, Alex. Physicochemical Profiling (Solubility, Permeability and Charge State). *Current Topics in Medicinal Chemistry*. 2001, 1, 277-351.
- 2.) Chait, A., Discovery ADMET Profiling: Solubility Technique. *Bioscience Tech.*, 2003.05: 33 – 34.
- 3.) ASTM: E 1148-02, *Standard Test Method for Measurements of Aqueous Solubility*, Book of Standards Volume 11.05.
- 4.) Prentis, R.A. *et al.* Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964-1985). *Br. J. Clin. Pharmacol.* 1988, 25, 387-396.
- 5.) Amidon, G. L.; Lennernas, H.; Shah, V. P.; Crison, J. R. A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of *In Vitro* Drug Product Dissolution and *In Vivo* Bioavailability. *Pharm. Res.* 1995, 12, 413-420.
- 6.) Oldenburg K, Pooler D, Scudder K, Lipinski C, Kelly M. High throughput sonication: evaluation for compound solubilization. *Comb Chem High Throughput Screen.* 2005 Sep;8(6):499-512.
- 7.) Yingqing Ran, Yan He, Gang Yang, Jennifer L.H. Johnson, Samuel H. Yalkowsky. Estimation of aqueous solubility of organic compounds by using the general solubility equation. *Chemosphere* 48 (2002) 487–509.
- 8.) Erik Rytting, Kimberley A. Lentz, Xue-Qing Chen, Feng Qian, and Srini Venkatesh. Aqueous and Cosolvent Solubility Data for Drug-like Organic Compounds. *The AAPS Journal* 2005; 7 (1) Article 10.
- 9.) Kasim, Nehal A. Molecular Properties of WHO Essential Drugs and Provisional Biopharmaceutical Classification *Molecular Pharmaceutics*, ASAP Article 10.1021/mp034006h S1543-8384(03)04006-1.
- 10.) Machatha SG, Sanghvi T, Yalkowsky SH. Structure Determination and Characterization of Carbendazim Hydrochloride Dihydrate. *AAPS PharmSciTech.* 2005; 06(01):
- 11.) Tomida H, Yotsuyanagi T, Ikeda K. Solubilization of steroid hormones by polyoxyethylene lauryl ether. *Chem Pharm Bull* (Tokyo). 1978;26:2832-2837.
- 12.) Yalkowsky, S.H. and R.M. Dannenfelser. 1992. *Aquasol Database of Aqueous Solubility*, Version 5. College of Pharmacy, Univ. of Ariz, Tucson, AZ. PC Version.

## Figures & Tables:

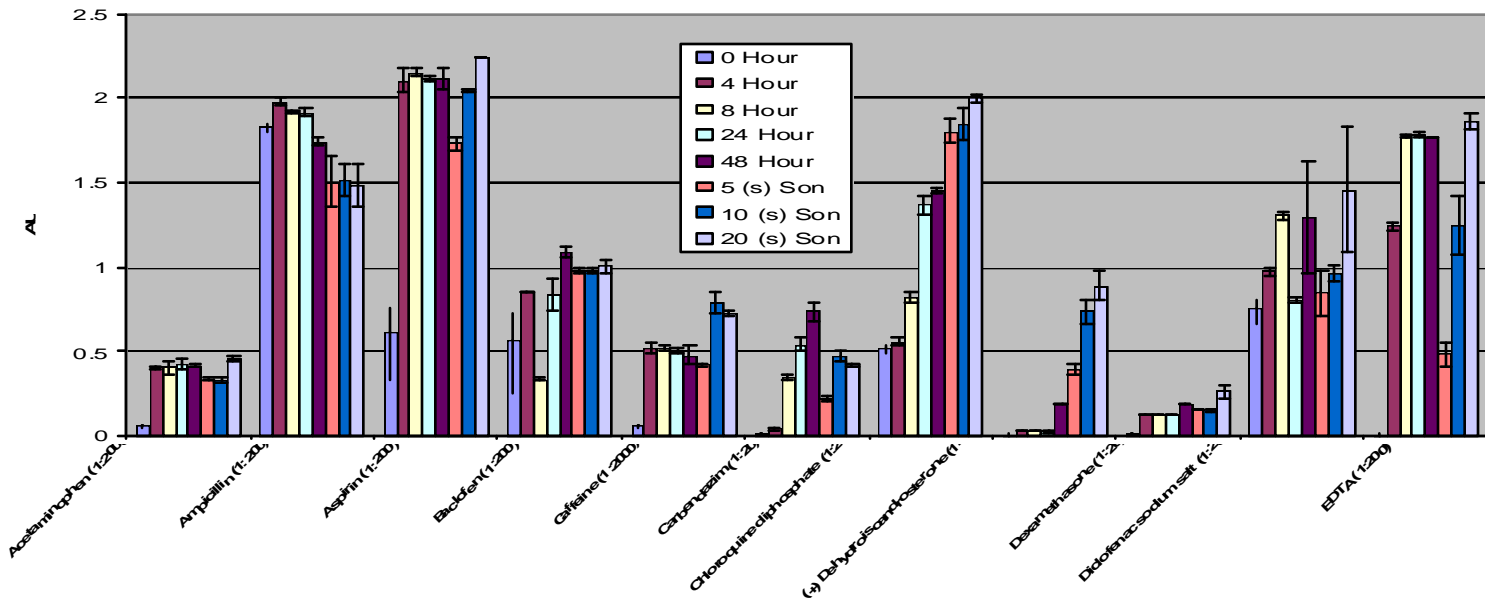
**Table 1: Compound Information**

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	-0.951	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	-0.71	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-	D6899	15307-	318.1	157	4.0		Acid	-5.097	8

	Aldrich		79-6							
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

• Aqueous solubility in mol/L

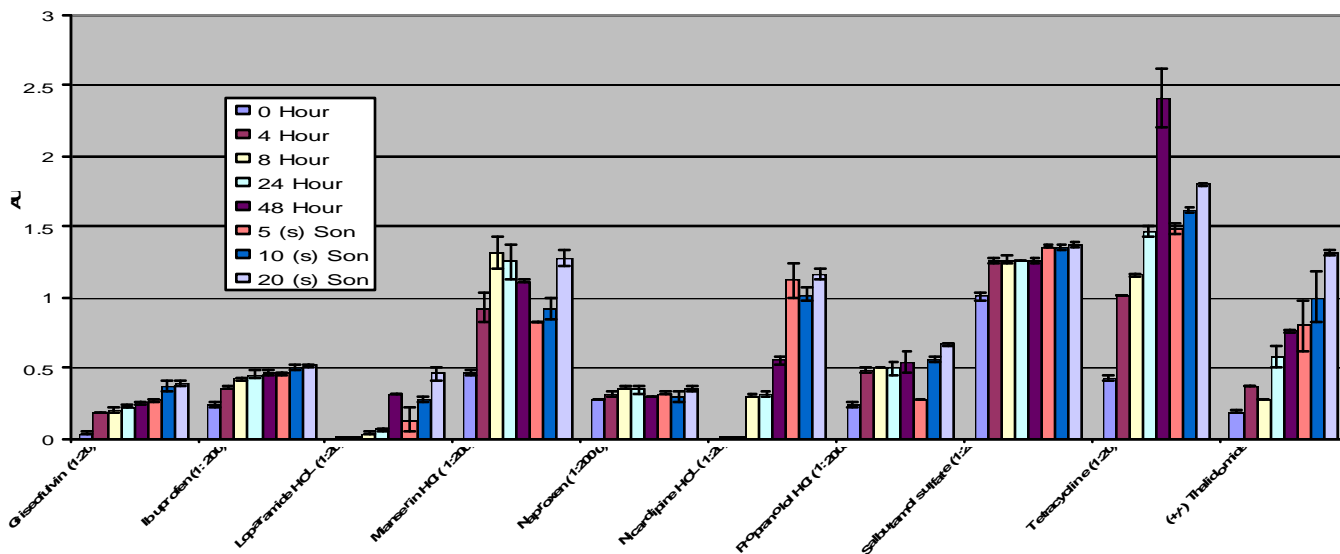
**Figure 3: Soluble Compounds I**



**Figure 3:**

Comparison of the dissolution of compounds with 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 of the 11 compounds showed more dissolution with 20 seconds of sonication than 48 hours of shaking while the remaining 3 were in good agreement.

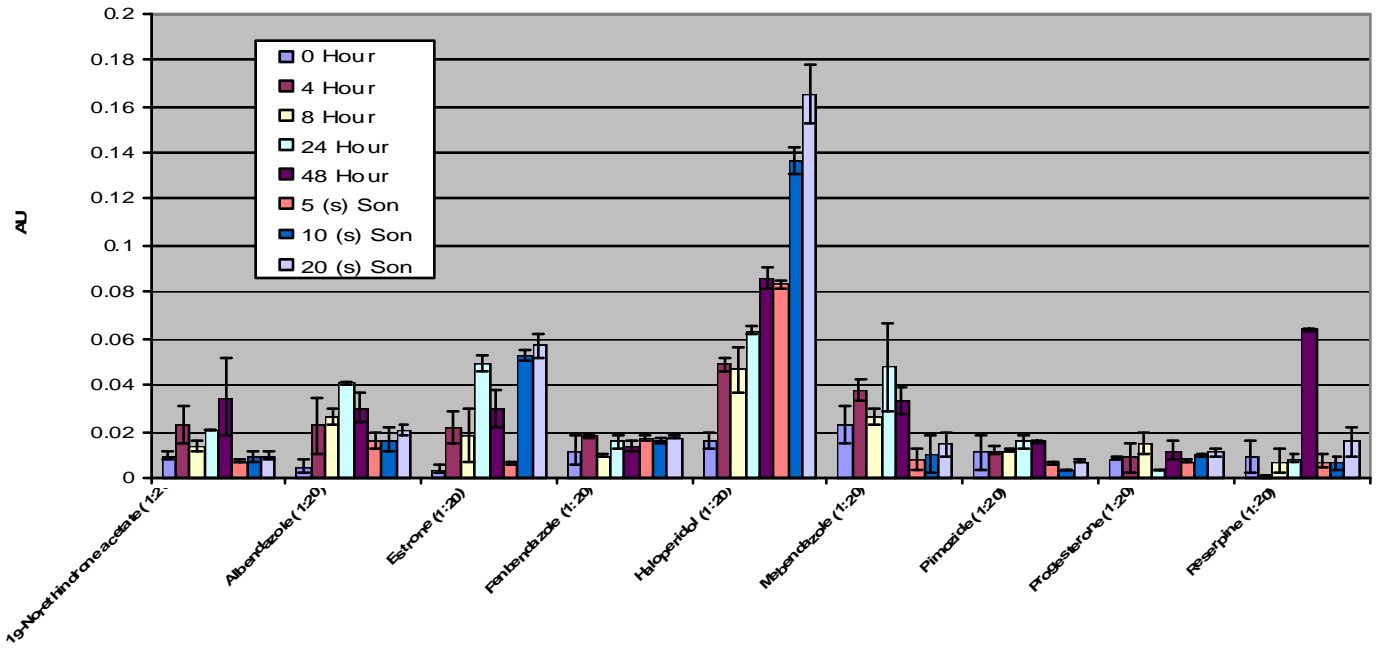
**Figure 4: Soluble Compounds II**



**Figure 4:**

Comparison of the dissolution of compounds with 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 of 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: Insoluble Compounds**

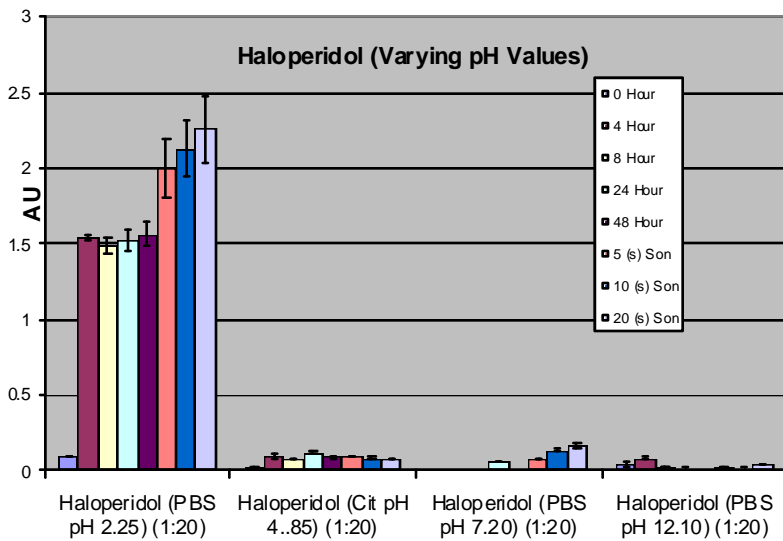


**Figure 5:**

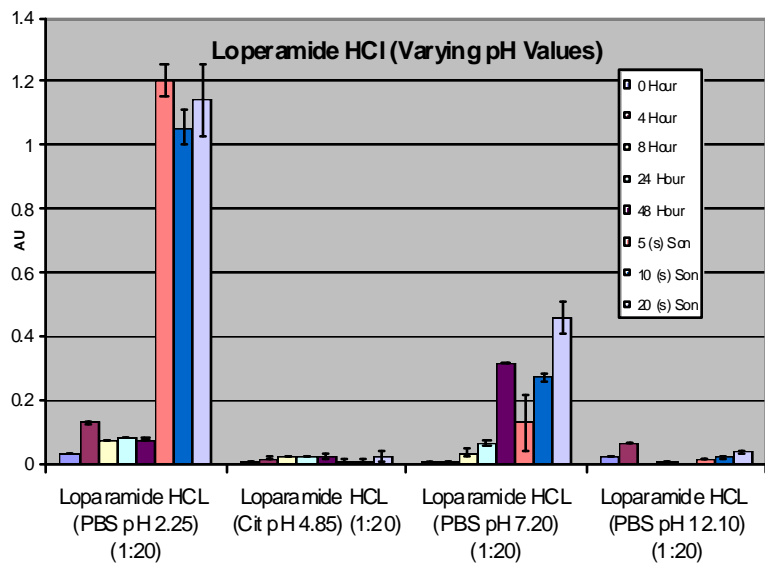
Results in this figure indicate as expected sonication while dramatically affecting the dissolution rate can not affect the thermodynamic equilibrium solubility.

**Figure 6A-6D:**

**Figure 6A:**

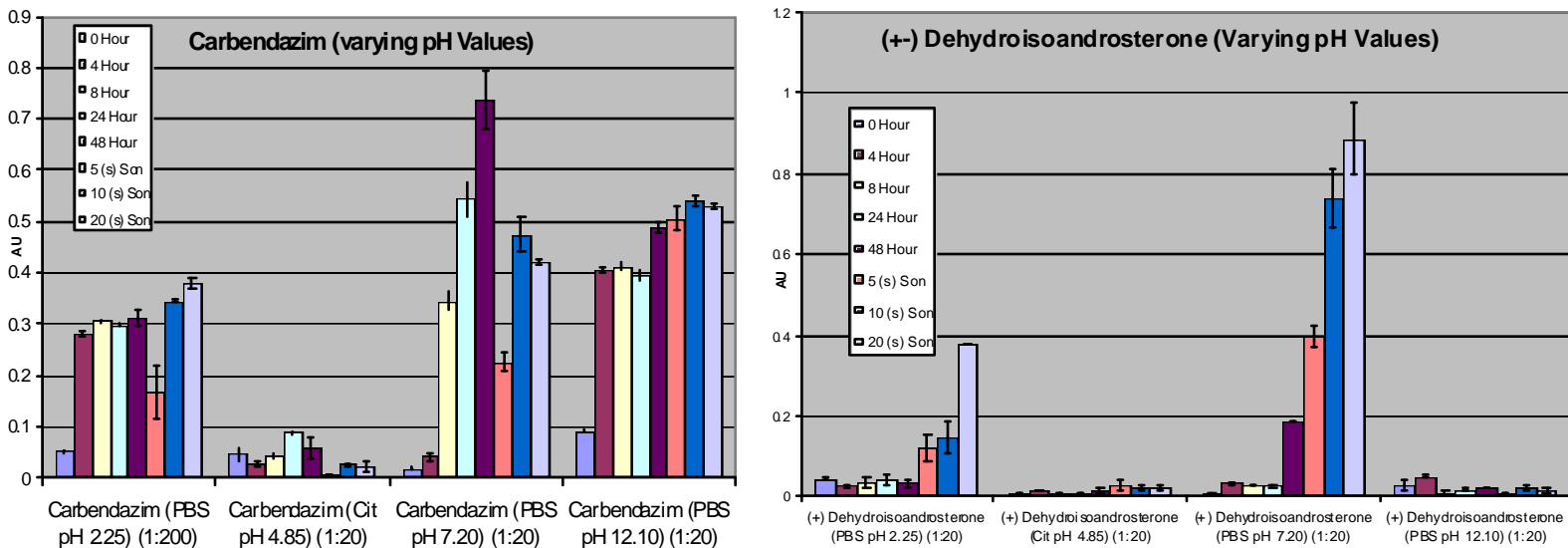


**Figure 6B:**



**Figure 6C:**

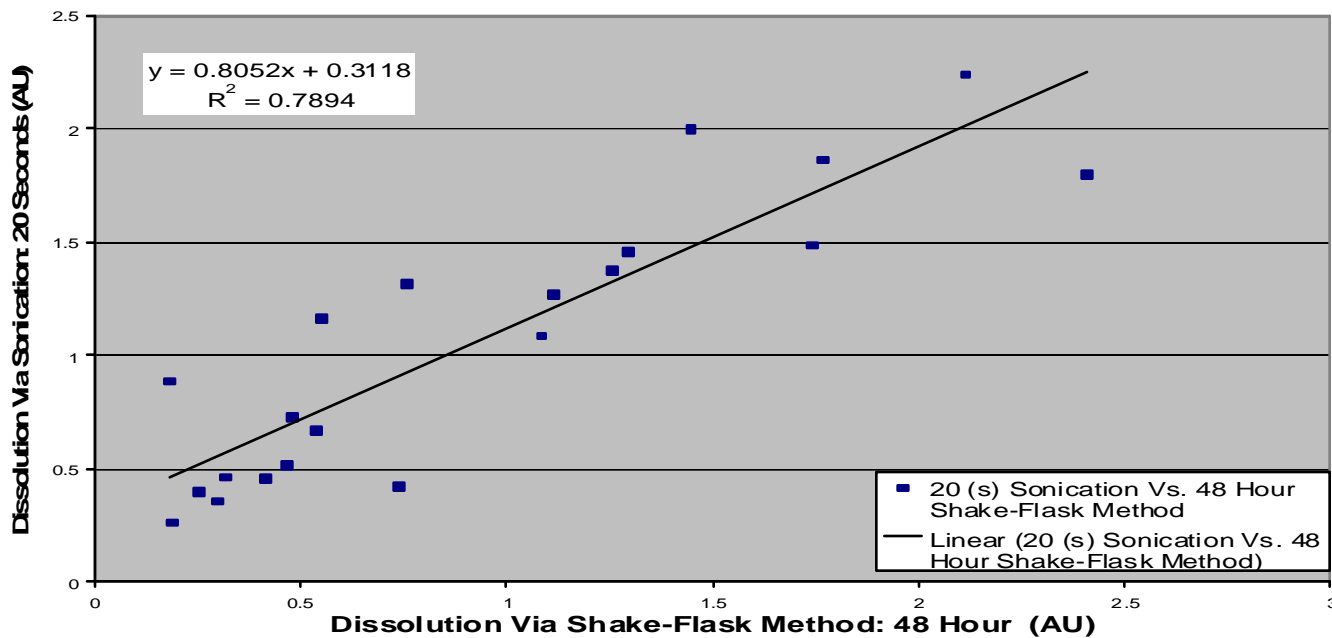
**figure 6D:**



**Figure 6A-6D:**

The data shows varying the pH of the solvent has no affect on the SonicMan’s compound dissolution capability as the SonicMan dissolves compounds to the same relative amount as the shake-flask method at different pH’s.

**Figure 7: SonicMan vs. Shake-Flask Correlation**



**Figure 7:** The solubility data obtained with 20 seconds of sonication via the SonicMan and with 48 hours of shaking via the shake-flask method is displayed. As shown, the SonicMan data is in good agreement with the shake-flask (the conventional method to get the thermodynamic solubility of a potential drug candidate in drug discovery) data. Each point in the figure represents an absorption value (20 sec Son. Vs. 48 Hr shake-flask) from 1 of the 30 compounds displayed in figures 3-5.

