

AQUEOUS SOLUBILITY

Poorly soluble compounds can dramatically reduce productivity in drug discovery and development.

Cerep's solubility assay is an efficient and reproducible high-throughput procedure designed to determine aqueous solubility of small organic molecules. Results from this test help our customers interpret *in vitro* assay results, recognize compounds that are solubility limited, and prioritize compounds.

■ ARE INSOLUBLE COMPOUNDS SLOWING DOWN YOUR DRUG DISCOVERY?

Modern screening technology has made it possible to identify increasingly more compounds with very low aqueous solubility (<10 μM) as hits. These compounds often become lead candidates in their respective discovery programs. However, smooth development of these compounds into drugs is often difficult. Discovery scientists are all too familiar with compounds "crashing out", i.e., precipitating during serial dilutions, in the screening buffer or cell culture medium after aliquots of the compound have been added from a stock solution in organic solvent. This ultimately leads to erroneous and/or irreproducible results. Extra time and resources are necessary to develop and test custom formulations for poorly soluble compounds in order to obtain meaningful drug exposure data during animal studies. This trial and error process is not always successful, and after much investment, some promising leads must be dropped altogether because adequate drug exposure for pivotal proof-of-concept studies cannot be obtained. Even if a preclinical formulation has been successfully developed, a time consuming reformulation may be required to obtain a formulation acceptable for clinical studies.

Potential complications arising from low aqueous solubility

- ▶ **Compound precipitation during:**
 - . serial dilutions in buffer
 - . biochemical assays
 - . functional assays
 - . cell-based assays
- ▶ **Reduced target specificity**
- ▶ **Low bioavailability in animal studies**

■ MORE TROUBLE AHEAD

If absorbed, compounds with low solubility are almost always highly bound to plasma proteins. This leads to slow tissue distribution and can result in drug-drug interaction during clinical trials. Research conducted at Cerep suggests that the target specificity of small organic molecules can diminish as solubility declines creating a high risk for the identification of false hits or leads. Additionally, "promiscuous" compounds (those with poor target specificity) can result in unpredictable *in vivo* effects.

■ HOW SOLUBLE ARE YOUR COMPOUNDS?

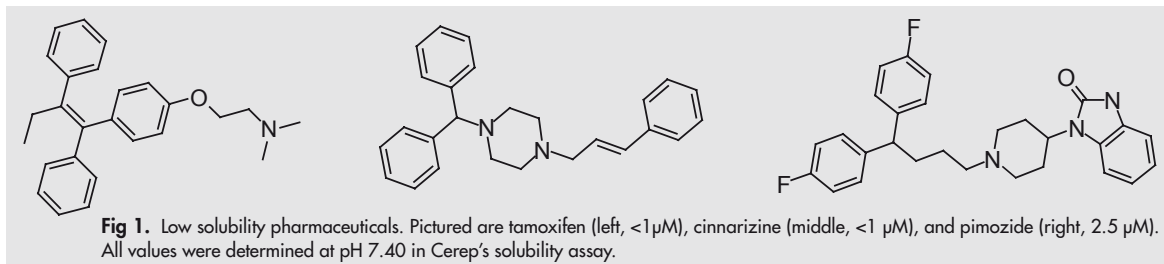
Studies at Pfizer Central Research have shown that lipophilicity and molecular weights of their medicinal chemistry products have steadily increased over time, thus decreasing aqueous solubility. The advent of combinatorial chemistry has accelerated this trend. Knowledge of the solubility of all precandidate molecules improves the interpretation of screening data and raises awareness for the great importance of drug solubility during lead optimization. This will help avoid the frustration associated with the challenges that insoluble compounds present during preclinical development.

■ HOW MUCH SOLUBILITY IS REQUIRED?

There are examples of successful drugs having very low solubility (e.g., those pictured in Fig. 1).

However, the vast majority of marketed pharmaceuticals analyzed at Cerep have much higher solubilities (see the table on next page). A good compound must be able to reach its target at effective concentrations. Therefore, the lowest acceptable solubility of a compound is related to its pharmacologic potency and its permeability.

At Cerep we believe that drug development is more efficient when compounds are not solubility limited. In our experience it is difficult to obtain reproducible screening results with compounds that have solubility less than 10x the apparent IC_{50} . We consider these compounds solubility limited. Low micromolar aqueous solubility (not uncommon with many of the experimental compounds studied at Cerep) can therefore be acceptable only for extremely potent and/or permeable compounds. However, many *in vitro* assays designed to determine compound selectivity, ADME properties, or toxic liabilities operate at concentrations of up to 30 μM and do not produce accurate results with insufficiently soluble compounds.



■ THERMODYNAMIC VS. APPARENT SOLUBILITY

It is important to differentiate the true thermodynamic solubility from the apparent solubility measured during drug screening. Thermodynamic solubility determination requires that crystalline compound be equilibrated with buffer. For drug discovery support, this slow process is unnecessary. At Cerep we measure the apparent solubility using conditions found in screening tests (drug dissolved in an

organic solvent, usually DMSO, is added to buffer). Under these conditions, insolubility is a consequence of crystallization which, like solubilization, is not an instantaneous process. Therefore, a well defined incubation period is required to obtain reproducible results.

■ CEREP'S SOLUBILITY ASSAY

We add compound dissolved in DMSO to isotonic buffer (pH 7.40) at a concentration of 200 μM (2% DMSO final concentration). After the incubation process, dissolved drug is measured by a chromatographic procedure with photodiode array detection. The principal goal of this assay is the rapid identification of compounds having very low micromolar solubility. The maximum solubility that can be determined is 200 μM and the limit of quantitation is often smaller than 1 μM depending on the absorbance of the compound.

► SMALL AMOUNT OF COMPOUND REQUIRED

The test can be run with as little as 100 nmol of compound. 1 mg of pre-weighed compound suffices.

► RAPID TURNAROUND TIME

The results are delivered within two weeks upon receipt of the compounds at the testing site of Cerep. Data are made available on line as soon as they are validated.

■ HIGH THROUGHPUT

The combination of a universal HPLC method with large bandwidth photodiode array detection provides fast and reliable results with virtually all small organic molecules that have drug-like structures. The entire process, including data analysis, is highly automated and scalable so that even large compound libraries can be analyzed in surprisingly short time frames.

■ PURITY INFORMATION AS AN ADDED VALUE

The chromatographic analysis also allows for an estimation of each compound's purity at the wavelength chosen for detection. This additional information is most useful when newly synthesized (not yet scaled up) compounds are analyzed. The customer report includes a chromatogram and a UV-VIS spectrum for each compound.

■ CUSTOMIZATION

The assay can be customized as per the customer's specifications. Some examples of customization include number of replicates, choice of buffer or water, and detection wavelength for purity analysis. Solubility can also be determined in simulated gastric and intestinal fluids (prepared according to UPS specifications). Results in these fluids can give an indication if solubility in the GI tract is a limiting factor for oral bioavailability.

■ QUALITY CONTROL

Each plate of customer samples includes eight reference compounds as internal controls. These compounds were selected for molecular diversity and have solubilities across the entire range of the assay (0-200 μM). Only plates whose reference compounds fall within specified ranges pass quality inspection.

Results of eight reference compounds are included with every analysis of customer compounds. Values reflect data collected over the course of five years in the aqueous solubility (PBS, pH 7.4) assay ►

	Mean (μM)	Low (μM)	High (μM)
metoprolol tartrate	187.2	173.6	222.3
rifampicin	183.5	171.0	222.1
ketoconazole	127.2	103.1	154.5
phenytoin	95.5	77.5	112.4
haloperidol	53.6	41.5	63.5
simvastatin	17.9	9.8	36.1
diethylstilbestrol	7.3	5.2	9.6
tamoxifen	1.3	0.3	3.6

Solubilities of various drugs as determined in Cerep's aqueous solubility (PBS, pH 7.4) assay ▼

Compound	μM Sol	Compound	μM Sol	Compound	μM Sol	Compound	μM Sol
acetaminophen	201.0	desipramine	195.9	hydrocortisone	202.9	progesterone	21.9
acetazolamide	198.1	dexamethasone	179.1	ibuprofen	205.0	propranolol	201.9
ajmaline	201.0	dextromethorphan	197.2	imipramine	180.6	quinidine	198.1
ampicillin	198.5	diclofenac	197.5	ketoconazole	152.3	ranitidine	203.5
atenolol	196.0	diltiazem	192.5	ketoprofen	203.4	reserpine	1.7
atropine	203.3	disopyramide	198.6	lidocaine	188.6	strychnine	203.4
bisoprolol	200.8	estrone	6.2	metoprolol	204.0	sulfaphenazole	199.9
caffeine	197.2	ethacrynic acid	200.2	mexiletine	203.0	sulfisoxazole	201.6
carbamazepine	197.1	flecainide	204.9	mianserin	68.1	terfenadine	152.8
cefotaxime	192.8	flufenamic acid	200.1	nicardipine	7.1	tetracycline	205.1
chloramphenicol	199.0	flunarizine	<1	norethindrone	32.9	thalidomide	55.2
chlorpheniramine	192.5	flurbiprofen	199.1	ouabain	203.1	tolbutamide	202.6
chlorpromazine	107.4	fluoxetine	172.4	paclitaxel	<1	trifluoperazine	44.7
cimetidine	193.2	furfurylline	198.7	phenacetin	202.8	verapamil	199.5
cinnarizine	<1	furosemide	200.5	pimozide	2.5	yohimbine	198.4
colchicine	196.4	griseofulvin	66.8	probenecid	197.5		



FRANCE
Le Bois l'Évêque
86600 CELLE L'ÉVESCAULT
tel. +33 (0)5 49 89 30 00

(Headquarters)
155 boulevard Haussmann
75008 PARIS
tel. +33 (0)1 45 64 44 60

USA
15318 N.E. 95th Street
REDMOND, WA 98052
tel. +1 (425) 895 8666

JAPAN
Namiki Shoji Co., Ltd.
Kenseishinjuku Bldg. 5-5-3
Shinjuku, Shinjuku-ku
TOKYO, 160-0022
tel. +81 (0)3 3354 4026
fax +81 (0)3 3352 2196

CHINA
Ai Di Sheng (Edison) Road 326,
302-1 room
Zhangjiang High-Tech Park
SHANGHAI
tel. +86 18702160370

■ QUESTIONS OR CONCERNS?

Please contact us: sales@cerep.com

sales@cerep.com
www.cerep.com