Poor Aqueous Solubility - an Industry Wide Problem in ADME Screening

Talking Points

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Slide 1
I am very pleased for the invitation to deliver this talk at the Spotfire user meeting April 25-26, 2002 Versailles, France.

Points to take home:

1) Physicochemical property changes in recent drugs makes finding orally active drugs more difficult. Poor solubility will be viewed as the predominant problem if leads come from high throughput screening. Poor permeability will be viewed as the major problem if leads arise from structure based design.

2) Adverse property changes can be managed through appropriate use of computational and experimental strategies.

3) Turbidimetric solubility should only be used in an early discovery setting and not in the development phase and not as one approaches candidate selection.

Slide 2
This 3D graph illustrates what can too easily happen if only potency is improved. It may be impossible to achieve oral activity (even with high potency) if solubility and permeability are very poor.

In theory, extremely high potency will solve a permeability or solubility problem. In practice, it is quite difficult to get orally active drugs at doses below 0.1 mg/kg. The reason is that at very low doses a variety of metabolic processes can easily destroy the drug. At higher drug doses, these metabolic processes are saturated and less important.

In our opinion, it is often easier to solve solubility problems than to solve problems in passive membrane permeability since the range in drug-like solubility is much greater than for permeability. For example, the FDA’s proposed bioequivalence classification system (BCS) classifies drugs into 4 classes depending on whether drugs have high or low permeability and high or low solubility. In the BCS, the range for permeability covers considerably less than three orders of magnitude while that for solubility covers a full six orders of magnitude.

The best way to solve a permeability or solubility problem is with chemistry. The key to avoiding this problem is to provide the chemist with information on solubility and permeability at the same time as the potency information is received.
Slide 3

This bar graph shows the trend in molecular weight for compounds synthesized in our medicinal chemistry labs (shown in red) and compounds purchased from external commercial sources (shown in blue).

In Groton we began HTS screening in 1989, and increased HTS screens through 1992. The percentage of compounds with a molecular weight over 500 (which we believe is undesirable) tracks exactly with the increased HTS screening. More and more of our leads were from HTS, these had poorer physicochemical profiles and when our medicinal chemists followed up these leads they made compounds with profiles like those of the leads or sometimes even worse than those of the leads.

The trends in compounds made in our medicinal chemistry labs are not aberrant, they are completely logical (and predictable) in terms of medicinal chemistry principles and the information available to the chemists. For example, introducing a lipophilic moiety (e.g. a methyl) so as to fit into a receptor is one of the best ways to improve in-vitro potency. This same change however, also increases lipophilicity.

Compounds purchased from commercial sources (in blue) were intended for random HTS screening and show no upwards trend in high MWT.

A bar graph with high lipophilicity instead of high MWT would look very similar.

Slide 4

The first 190 of our high throughput screening (HTS) hits were more lipophilic than phase II or marketed drugs as measured by the Moriguchi LogP calculation.

As a caveat, I want to point out that these HTS hits were those highlighted as the best hits in summaries and reports. They were the result of both the primary and secondary screening processes (a technical issue) and an evaluation as to chemical suitability for lead pursuit (a people issue).

The people issue is particularly important because chemists have traditionally focused on in-vitro optimization. This past history can lead to a bias in which compounds are assessed by the probability that in-vitro activity can be improved. This can lead for selection of larger compounds with more room for synthetic exploration of SAR but with poorer physicochemical properties.
Slide 5
We looked at distinctly different libraries to try to determine whether recently made medicinal compounds are different from older drugs.

The library of 7400 INN/USAN and marketed drugs was our benchmark. This is a significant fraction of all drugs that have reached phase 2 status. For example, there were about 9,500 USAN drugs listed in the most recent publication of the US Pharmacopeia.

To get at the properties of newer compounds we used the following process.

From the Derwent World Drug Index we searched for compounds abstracted in 1997, 1998 and 1999 with the text string “trial-prep” in the mechanism of action field. These compounds were too new to have received INN or USAN names and were too new for Derwent to have abstracted the Chemical Abstracts Registry Number.

Slide 6
This graph shows the distribution of molecular (formula) weights of four classes of compounds.

Shown in yellow are compounds appearing in the Derwent World Drug Index. This includes a very wide range of compounds. All have some sort of biological activity but most are not “drug like”.

Shown in blue are drugs with International Non Proprietary Names (INN) and United States Adopted Name (USAN) name. These names are generally given when a compound enters phase II studies. These are the compounds that have survived phase I with sufficient oral bioavailability and acceptable pharmacokinetic and pharmacodynamic parameters.

Shown in red are recently disclosed potential drugs that were abstracted by Derwent in 1996 and 1997 from the medicinal chemistry journal and conference literature. The vast majority of these are too new for the Chemical abstracts registry number to appear in databases and none of these compounds have INN or USAN names.

Shown in green are New Chemical Entities (NCE). These are the drugs that actually reached market and are the compounds that are summarized in the “To Market - To Market” chapter in the back of the issues of “Annual Reports in Medicinal Chemistry.”

The MWT corresponding to the 90th percentile and a decreasing probability of oral activity is marked by a horizontal line.
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Newer drugs also are more lipophilic than older drugs but the difference is not as great as for size.

I think that the reason for this, is that newer drugs tend to be a mixture of compounds made in pursuit of HTS leads (which strongly tend to be more lipophilic) and compounds with peptido mimetic-like character which tend to be larger and have more hydrogen bond donor and acceptor groups but which are NOT excessively lipophilic.

This will be shown more clearly in subsequent slides.

Slide 8
The distribution of parameters for INN/USAN drugs can be used to define a property range where oral activity is increasingly difficult due to poor absorption or poor permeability. All the curves exhibit a leveling as parameters reach unfavorable values for oral activity. The 90th percentile of each parameter is shown by the arrows.

The colored lines show the distribution of:
- MLogP - lipophilicity (in blue) as measured by the Moriguchi Log P algorithm
- NWT/100 - molecular weight (in light green), divided by 100 for plotting
- OH+NH - the sum of OH plus NH (in red) as an index of H-bond donors
- O+N - the sum of oxygen plus nitrogen (dark green) as an index of H-bond acceptors

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This analysis led to a simple mnemonic which we called the “Rule of 5” because the parameter cutoffs all contained 5’s.

Namely that poor absorption or permeation are more likely when there are:
- More than 5 H-bond donors
- The MWT is over 500
- The CLog P is over 5 (or MLOGP is over 4.15)
- The sum of N’s and O’s is over 10
- Substrates for transporters and natural products are exceptions

Although this rule is very simple, it works remarkably well provided you understand its limitations.

First, it only works because the physical property profile of medicinal compounds being currently made is quite far outside that of marketed drugs.

Secondly, it doesn't work for compounds that are of natural product origin or have structural features originally derived from natural products, for example antibiotics, antifungals. The likely reason is the important roles of transporters in these classes.
The General Pharmaceutics Laboratory in our development organization profiles all newly nominated candidates. As part of the evaluation, a minimum acceptable dose (MAD) is calculated for oral dosage forms based on the expected clinical potency, the solubility and the permeability. This calculation serves to confirm that either the physicochemical properties of the candidate are easily within the acceptable range or that the properties lie within a difficult range that will require more than the average pharmaceutics manning.

We have adapted this calculation to create a simple bar chart that we distribute to our medicinal chemists. It answers the question of “how much solubility do I need?” The three middle bars describe the most common clinical potency that we encounter; namely that of 1 mg/kg. If the permeability is in the middle range as for the average heterocycle (the purple bar) then a thermodynamic solubility of about 50 ug/mL at pH 6.5 or 7 is required. If the permeability is low (as in a typical peptido-mimetic) the solubility should be about 200 ug/mL.


The logic behind the use of a turbidimetric solubility assay in early discovery to assist in achieving oral activity depends heavily on differences in solubility usage in early discovery as opposed to development settings.

We believe that a turbidimetric assay provides information that is more relevant in helping the chemist to make compounds that will show oral activity in early discovery biology assays. In discovery, no one equilibrates a well characterized solid for 24 or 48 hours in buffer solution, separates the phases, quantitates the concentration in solution prior to orally dosing an animal with the solution.

In early discovery, a compound that is not soluble per se or is not soluble by reasonable pH adjustment is dissolved in DMSO to make a concentrated solution. The DMSO solution is then injected into a well stirred gavage vehicle and an appropriate volume of vehicle is then administered by gavage as quickly as practical to get the compound into the animal before precipitation can occur. The content of DMSO is kept to a minimum, typically about 1% or less (the range below which there is minimal damage in cell culture).

Our turbidimetric assay mimics early discovery biology dosing in:

1) the addition of a concentrated drug in DMSO solution to aqueous vehicle
2) the tens of minutes time scale and
3) a final content of DMSO of less than 1%.
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The schematic for our flow cell turbidimetric solubility assay shows key steps.

A robot aspirates compound in DMSO from autosampler vials contained in an 8 by 12 plate format. The aspiration needle is dipped in methanol to remove any DMSO adherent to the exterior of the needle. The drug in DMSO is then added in 0.5 uL increments to a stirred mixing chamber containing pH 7 phosphate buffer. The volume of buffer in the mixing chamber and associated tubing is 2.5 mL so that the maximum DMSO content is 0.375% if enough drug in DMSO is added to reach the greater than 65 ug/mL upper solubility limit of the assay.

After each drug in DMSO addition, the aqueous is pumped to a modified HACH nephelometric turbidity unit (NTU) light scattering detector. NTU readings are taken at one minute intervals. A compound is out of solution if three consecutive readings are 0.075 NTU’s above the NTU before compound addition.

To save time, we truncate the assay if there are thee NTU readings above our baseline. After each assay, a series of aqueous and methanol rinses brings the solubility robot back to starting condition for the testing of the next compound.

Half plates of 45 compounds are always complete in 24 hours or less so that the robot will be ready for the next days run.

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Shown here is our experimental flow cell solubility assay. To assay a single compound takes between 7 and 25 minutes depending on when the compound precipitates. We quantitate light scattering using a highly modified HACH nephelometric detector. The flow cell assay is unaffected by colored impurities. Highly fluorescent compounds (emitting in the fluorescein range) do not generate a false positive signal. A cutoff filter blocks fluorescence emission below the low 600 nm range. We can use a filter because the light scattering detector has adequate sensitivity at the higher wavelengths.

This assay is our front line assay and is intended to measure solubility for compounds which do not have permeability problems.

Our turbidimetric solubility assay always gives higher solubility values than a thermodynamic solubility assay. In our experience, poor turbidimetric solubility (<= 20 ug/mL in pH 7 non-chloride containing phosphate buffer at ambient temperature) means that a compound has little or no chance for oral activity unless it has something else going for it, for example, exceptionally good permeability or very high potency.
Slide 14
In our prototype flow cell solubility instrument, baselines are very stable in the absence of precipitation.

The assay is designed to measure solubility values of <5 to >65 ug/mL in non chloride containing pH 7 buffer at ambient temperature. This solubility range covers the range that is needed for typical heterocyclic compounds without permeability problems and with a projected clinical oral potency of about 1 mg/Kg.

In this screen capture, precipitation occurs late. This is a typical pattern for a compound that is insoluble because of excessive lipophilicity.

Compounds that are poorly soluble because of high crystallinity typically precipitate very quickly and generate a stronger NTU signal when the solubility is exceeded.

Slide 15
Shown in this slide is the mixing chamber in our turbidimetric flow cell solubility assay. Here the compound in DMSO is added in 0.5 uL increments to well stirred, non chloride containing 0.05 M pH 7 phosphate buffer. The buffer strength is more than adequate to maintain constant pH even with samples that contain excess acid (or less commonly base). The maximum DMSO content is 0.375% if a solubility of greater than 65 ug/mL is attained.

The 2.5 mL of aqueous is pumped to a cell for reading by a modified HACH nephelometric turbidity unit (NTU) light scattering detector. The solubility apparatus is remarkably stable and our initial concerns about precipitate possibly clogging the plumbing tubing or fouling the reading cell proved groundless.

We periodically recalibrate our three solubility robots using commercially available NTU standards to make sure that the response and baselines are similar across the robots.

Slide 16
Flow Cell Turbidimetric Solubility Assay (cont.).

Two flow cell solubility robots run below maximum capacity with an actual output of 10,000 assays over the last year. We have recently added a third robot to provide more flexibility for further assay development.

If a compound is soluble at the upper limit of the flow cell assay it is automatically tested at a higher solubility range in a plate reader assay.

Our solubility assays are very manning sparing once the initial investment in instrument building and software development has been made. A single laboratory assistant runs both flow cell and plate reader assays for all of Groton discovery research with some time left over for other work.
The turbidimetric flow cell assay is highly reproducible when tested using the same compound.

Assay reproducibility was investigated by examining 1479 solubility assay results consisting of replicates of three or more on 444 compounds. The distribution of replicate assays is shown below:

- 6  6 to 9 replicate assays
- 12  6-replicate assays
- 29  5-replicate assays
- 49  4-replicate assays
- 348  3-replicate assays

The mean standard deviation in solubility across all 444 compounds was 2.73 ug/mL.

The assay standard deviations (STDEVS) are quite low at the solubility extremes, 2.42 ug/mL at the lower limit and 0.30 ug/mL at the upper limit. In the middle solubility ranges the STDEVS are higher. This is likely due to inter-assay variation in the time required for the compound to precipitate.

The percent false poor solubility was 4.6% based on 262 turbidimetric assays on 100 neutral or salt form compounds. Most of the false poor solubility was due to a few very reproducible replicate turbidimetric solubility assays. This suggests a compound related rather than assay variability cause. The cause may be due to differences in salt solubility between the salt form tested in the thermodynamic assay compared to that tested in the turbidimetric assay. As a result the percent false poor solubility is likely overstated. For example the 5 ug/mL turbidimetric solubility for the copper salt of piroxicam compared to the 460 ug/mL thermodynamic solubility for piroxicam was classified as false poor solubility.

The percent false good solubility was 17% based on 196 turbidimetric assays on 82 compounds. One half of the false good solubility was due to very reproducible replicate turbidimetric solubility assays. This strongly suggests that the cause is compound related. The most likely cause is a rate of compound precipitation that is slow relative to the 20 minute assay time scale. This type of behaviour will also occur in a typical early discovery biology oral dosing and therefore is not a “false” result relative to in-vivo SAR.

Compounds with false good solubility were very significantly associated with high lipophilicity and those with false poor solubility with low lipophilicity. Lipophilic (greasy) compounds tend to precipitate more slowly hence there is a higher probability that their solubility will be overestimated.
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We use our turbidimetric solubility assay in a 96 well plate reader if a compound has greater than 65 ug/mL in our flow cell assay or if calculations at the time of compound registration suggest that a permeability problem is likely and that therefore a higher solubility range is required.

This assay is designed to measure solubility in the 50-500 ug/mL range. We measure in the 50-250 ug/mL range as a follow on to the flow cell assay and at 100-500 ug/mL if we suspect a permeability problem. These ranges span the solubility required for poorly permeable peptido-mimetic like compounds.

Samples in DMSO are added to 200 uL pH 7 phosphate buffer in Spectra Image 96 well plates. UV Absorption is read at 450 nm with a Tecan SLT Spectra Image plate reader in a 5 by 5 matrix format. Each absorption element in the 5 by 5 matrix is blanked versus the corresponding element prior to buffer addition. Our lower detection limit of 0.2 NTU’s turbidity corresponds to 0.018 absorbance units. Signal processing (if necessary) is automatically performed by the analysis software.

Adding compound in DMSO to 200 uL buffer in the plate wells is non trivial because we cannot afford to waste compound. Both the previously described flow cell assay and this plate reader assay run on the same 1 mg of compound dissolved in 50 uL of DMSO. Our smallest addition aliquot is 500 nL which we can add with coefficients of variation of 5-7 percent using specially designed septum piercing needles.

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The flow cell assay and the plate reader assay are closely integrated. We receive electronic files from our compound management system (CMS) along with the compounds in autosampler vials in the actual plates used in both assays. These files from our CMS are used to set up the DMSO additions and dispensing of drug in DMSO to the pH 7 phosphate buffer in the flow cell assay. The software keeps track of the solubility results and if the solubility is greater than 65 ug/mL an electronic input file for the follow on plate reader assay is created.

The same plate and vials used in the flow cell assay are then transferred to a new robot which delivers four concentrations of each compound that exceeded 65 ug/mL solubility in the flow cell assay to the 200 uL of pH 7 buffer in each of the wells in the 96 well plate. This is the step illustrated in the graphic.

Rapid data reporting with minimal operator time are essential to success. Both assays generate hard copy legal auxiliary record pages that meet our records management standards. Both assays generate electronic output files that are uploaded daily to our Oracle databases. As soon as the data is uploaded, a variety of calculated properties are added to the Oracle database fields. These are then immediately available to our scientists, usually through their project ISIS/Base database views.
Slide 21
About one third of the compounds we test have poor solubility (<= 20 ug/mL) in our flow cell solubility assay. High lipophilicity is the best predictor of poor solubility. About 40% of experimentally insoluble compounds have Moriguch Log P (MLogP) greater than our cutoff value of 4.15. Over 80% of these are very likely to be poorly soluble. Very few experimentally highly soluble compounds (> 65 ug/mL) have MLogP greater than 4.15 (leftmost green bar).

More than half the poorly soluble compounds are not identified by the MLogP calculation. To provide SAR on these we have to run the solubility experiment. These poorly soluble, non lipophilic compounds precipitate because of high crystallinity. It is fortunate that these types of compounds are the easiest to detect in our assay because these are also the most difficult to rescue through formulation approaches.

Slide 22
The success of an intervention strategy to improve compound physicochemical properties should be quantifiable.

Using our computational “Rule of 5” alert in the last year and a half we have evidence for a reversal of some of the poorer physicochemical properties in our medicinal chemistry compounds that occurred prior to our intervention.

The change in the types of screening targets may make it very difficult or impossible to get back to the physicochemical profile in the pre HTS era.

However, if we succeed in keeping the current physicochemical profile we will continue to have the benefits of HTS while minimizing one of HTS’s major drawbacks.