PhysChem FORUM

Classification of ionisable drugs by apparent dissolution and precipitation rates

Jon Mole
Sirius Analytical
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- Principle of the CheqSol method
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- Future research and conclusions

Introduction

Recent investigations at Sirius using our CheqSol solubility assay has given us some interesting insights into the relationships between dissolution and precipitation rates for a range of drugs.

Our latest research indicates that molecules can be placed into one of four classes: *Chasers*, *Non-Chasers*, *Super-Chasers* and *Ghosts*.

Introduction to CheqSol

- Unique method for solubility measurement
- Runs on Sirius GLpKa / PCA200 instruments
- Requires pK_a value
- Uses “Chasing Equilibrium” process to determine intrinsic solubility

Sirius definitions of solubility

- **Kinetic Solubility** is the concentration of a compound in solution at the time when an induced precipitate first appears

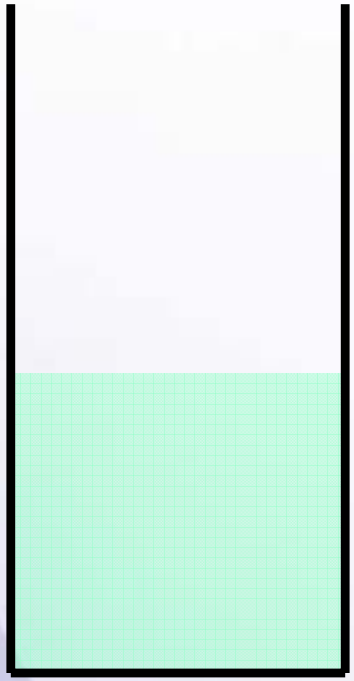
- **Equilibrium Solubility*** is the concentration of compound in a saturated solution when excess solid is present, and solution and solid are at equilibrium

- **Intrinsic Solubility** ** is the equilibrium solubility of the free acid or base form of an ionizable compound at a pH where it is fully un-ionized

*** also called Thermodynamic Solubility**

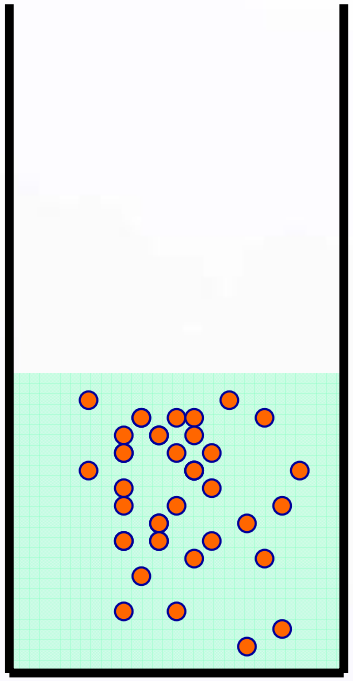
**** Hörter, D.; Dressman, J. B. *Adv. Drug Deliv. Rev.*, 1997, 25, 3-14**

Starting the CheqSol Assay - Seeking precipitation

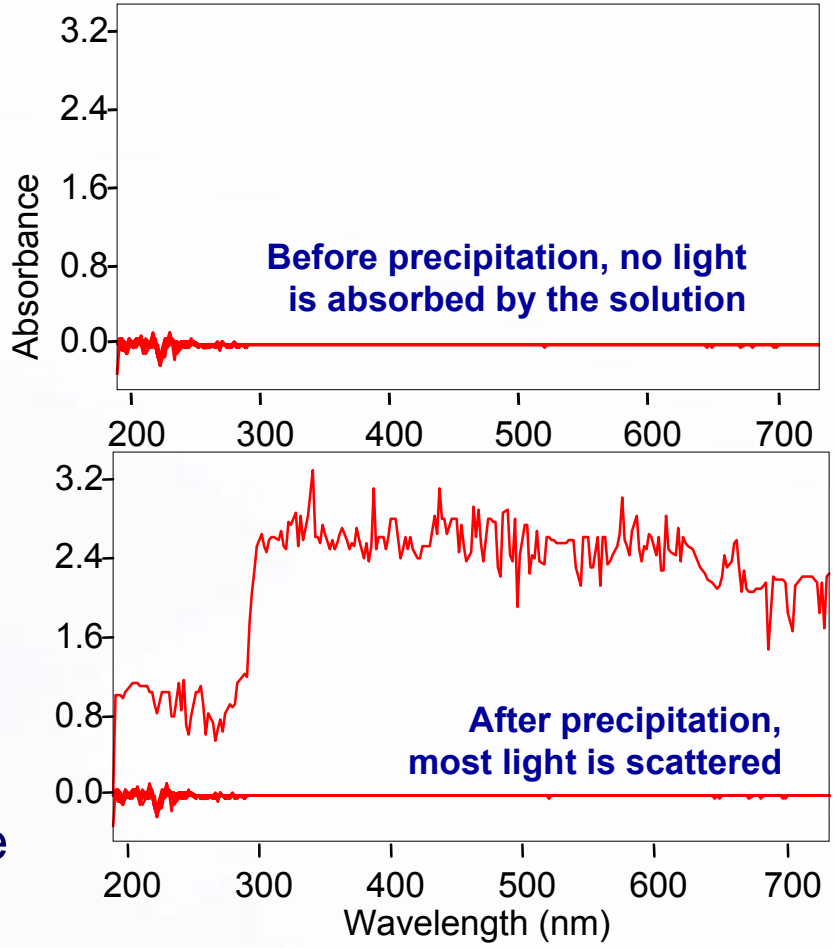


Solid (5 to 20mg) added to vial.

Instrument adds water (or water-cosolvent), then adjusts pH to dissolve sample.



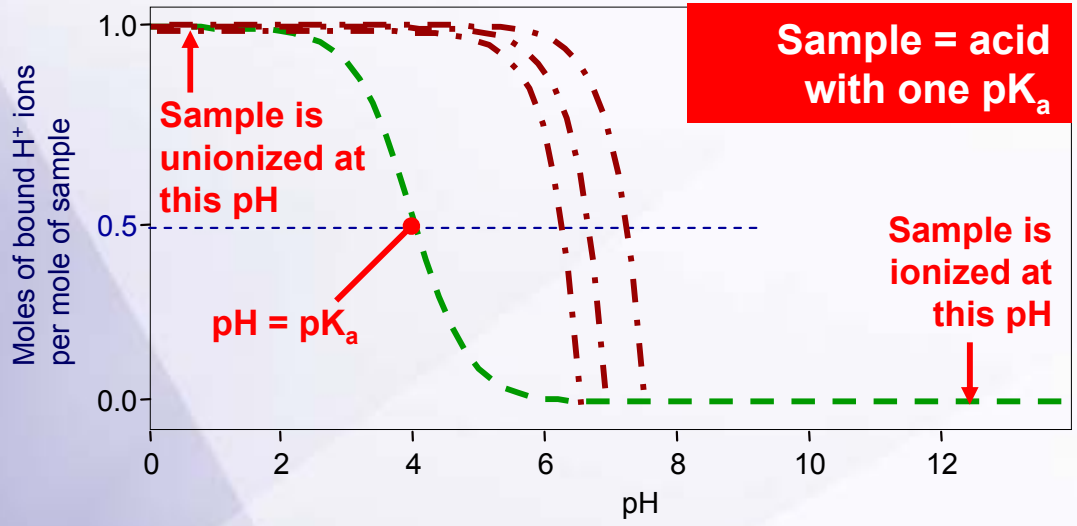
Solution titrated towards the pH where the sample becomes neutral. Eventually it precipitates

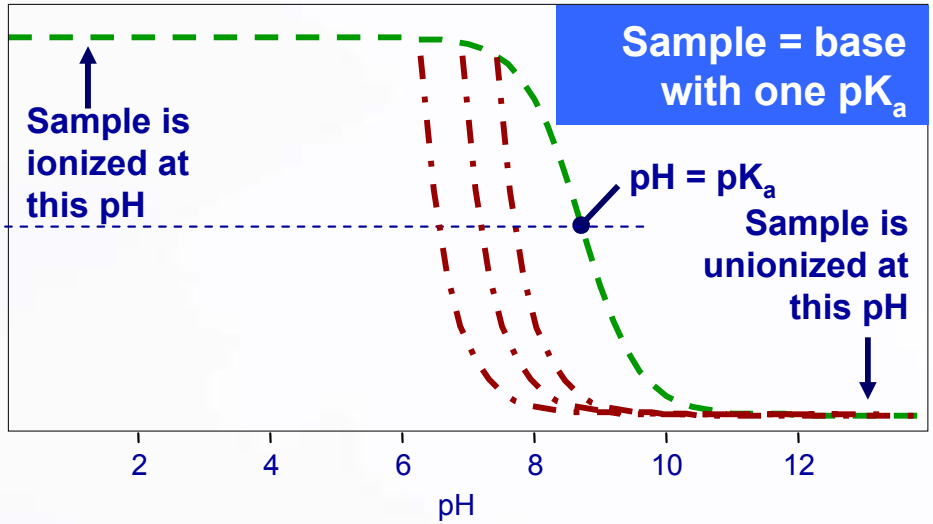


Precipitation causes light scattering, and D-PAS detects this as an increase in the light absorbed. Kinetic solubility determined at point of precipitation.

Bjerrum Graph - a very powerful tool

- Bjerrum Graphs are calculated from pH, pK_a and intrinsic solubility
- The Solution Bjerrum Graph (green line) represents an ionizable sample that is in solution across the entire pH range
- The Precipitation Bjerrum Graph (brown line) represents a sample when precipitate is present, and *solution and precipitate are at equilibrium*

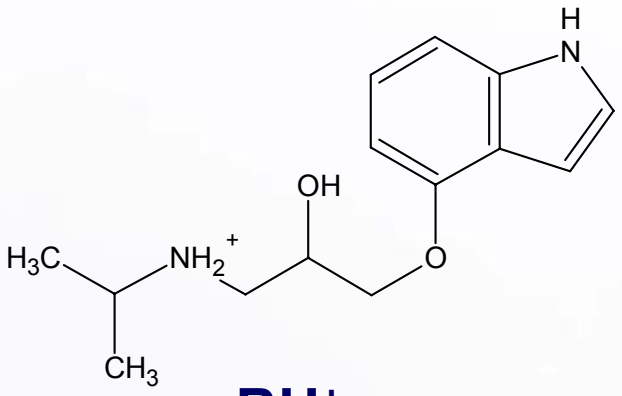




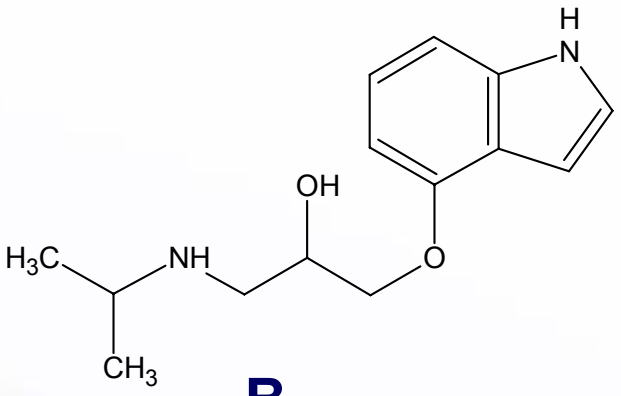
Solution Bjerrum Graph, depends on aqueous pK_a

Precipitation Bjerrum Graph. Greater shifts in pK_a caused by:
lower solubility (for a given concentration, shift increases as solubility decreases)
higher concentration (for a given solubility, shift increases with concentration)

CheqSol example – solubility of Pindolol (a chaser)



BH^+ soluble

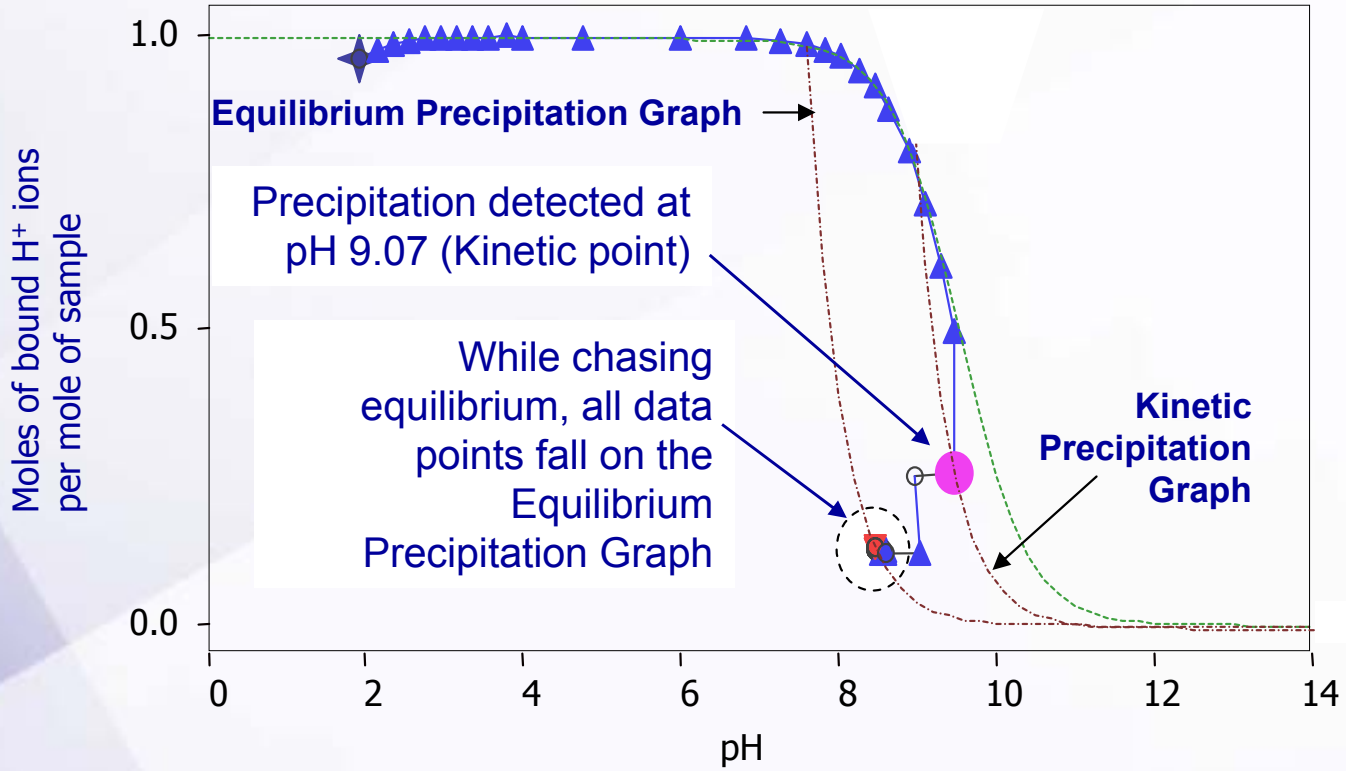


B insoluble

- Pindolol is a beta-blocker, used to reduce hypertension
- It's a secondary amine with pK_a of 9.54 (25°C, 0.15M ionic strength)
- The neutral form B is poorly soluble in water at high pH
- The ionized form BH^+ is soluble at low pH

Pindolol - Chasing method

- For Pindolol, the kinetic solubility falls on a different Precipitation Bjerrum Graph to the rest of the data
- Intrinsic solubility is determined from the data points on the Equilibrium Precipitation Graph, as explained in the following slides
- Kinetic solubility is higher than Intrinsic solubility
- Assay took 37 minutes to measure kinetic and Intrinsic solubility



Overview of Chasing Equilibrium

- **CheqSol** adds HCl or KOH solution while precipitate is present, and records the rate of pH change*
- This forces the neutral species to cycle between two states:

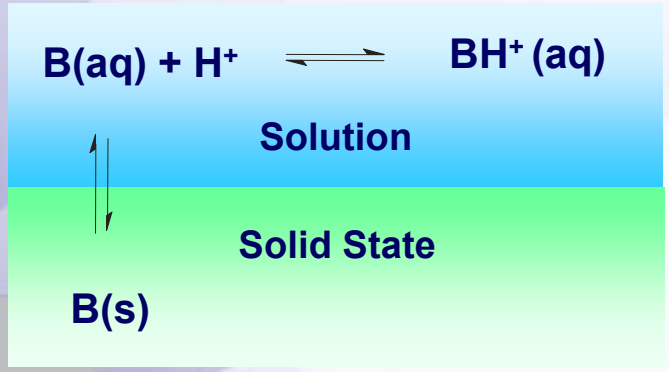
  supersaturated (excess neutral species in solution)
  subsaturated (excess undissolved neutral species)

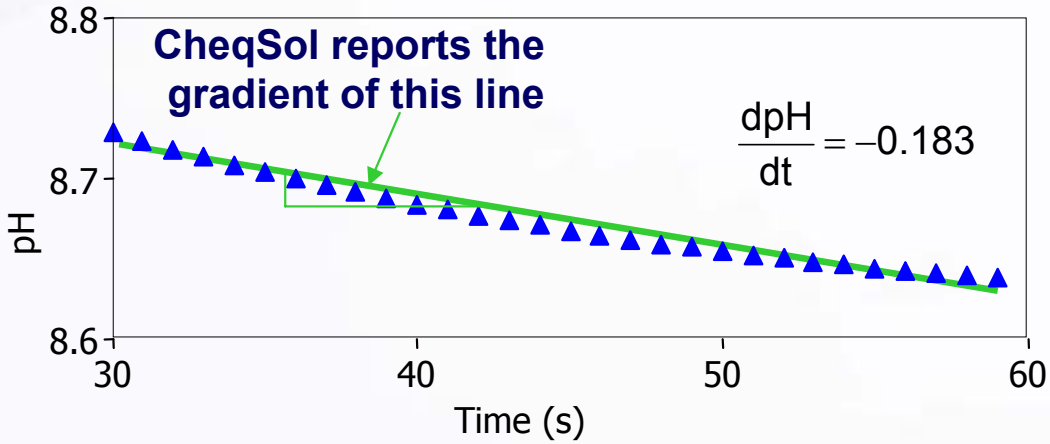
- Between these states, a point will be crossed where the concentration of neutral species would be at equilibrium
- This technique is called Chasing Equilibrium
- NOTE: **CheqSol** is short for Chasing equilibrium Solubility
- CheqSol was invented in April 2004 at Sirius by Martin Stuart and Karl Box. Sirius have applied for a patent for CheqSol

***after waiting until the onset of sustained response**

Supersaturated Pindolol

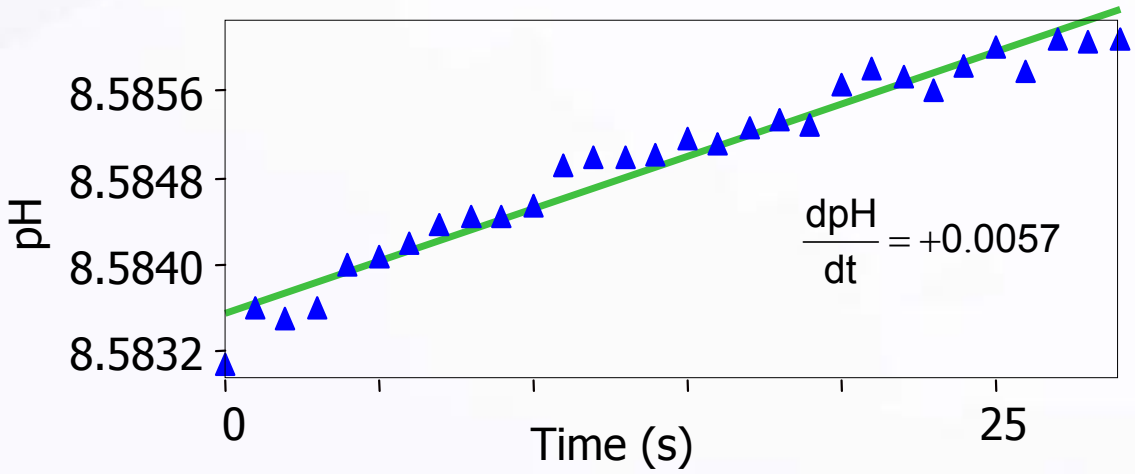
- When KOH is added to raise the pH, the solution of Pindolol becomes supersaturated before it precipitates
- When precipitate first appears there is a good deal of dissolved unionized material B, but it could take hours before it all falls out of solution
- KOH addition pauses when precipitation has been detected
- Molecules of B interact to form particles of precipitate, and BH^+ ions convert to B to replace some the B that was lost.
- This releases H^+ ions, and the pH goes down



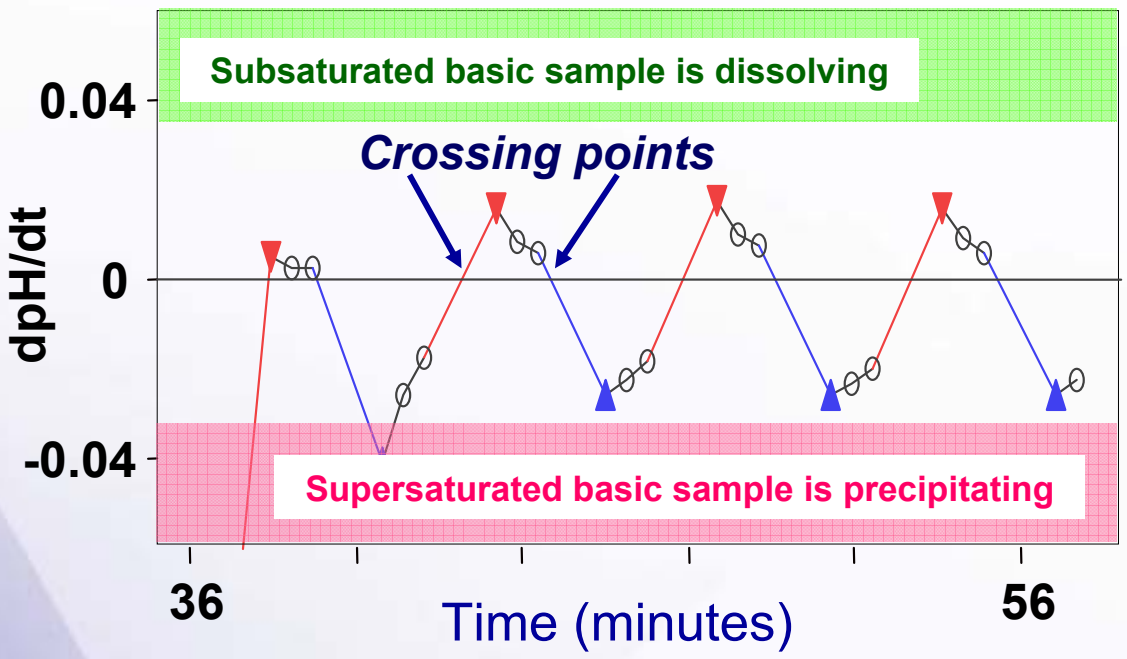


Subsaturated Pindolol

- After recording the linear fit to the gradient, CheqSol adds HCl to lower the pH.
- Some of the dissolved B converts to BH^+ in solution
- The solution becomes subsaturated, i.e. there is an excess of precipitate that could dissolve
- B dissolves. Some of it converts to BH^+ in solution. This consumes H^+ ions, and the pH goes up



The Crossing Point Graph for Pindolol

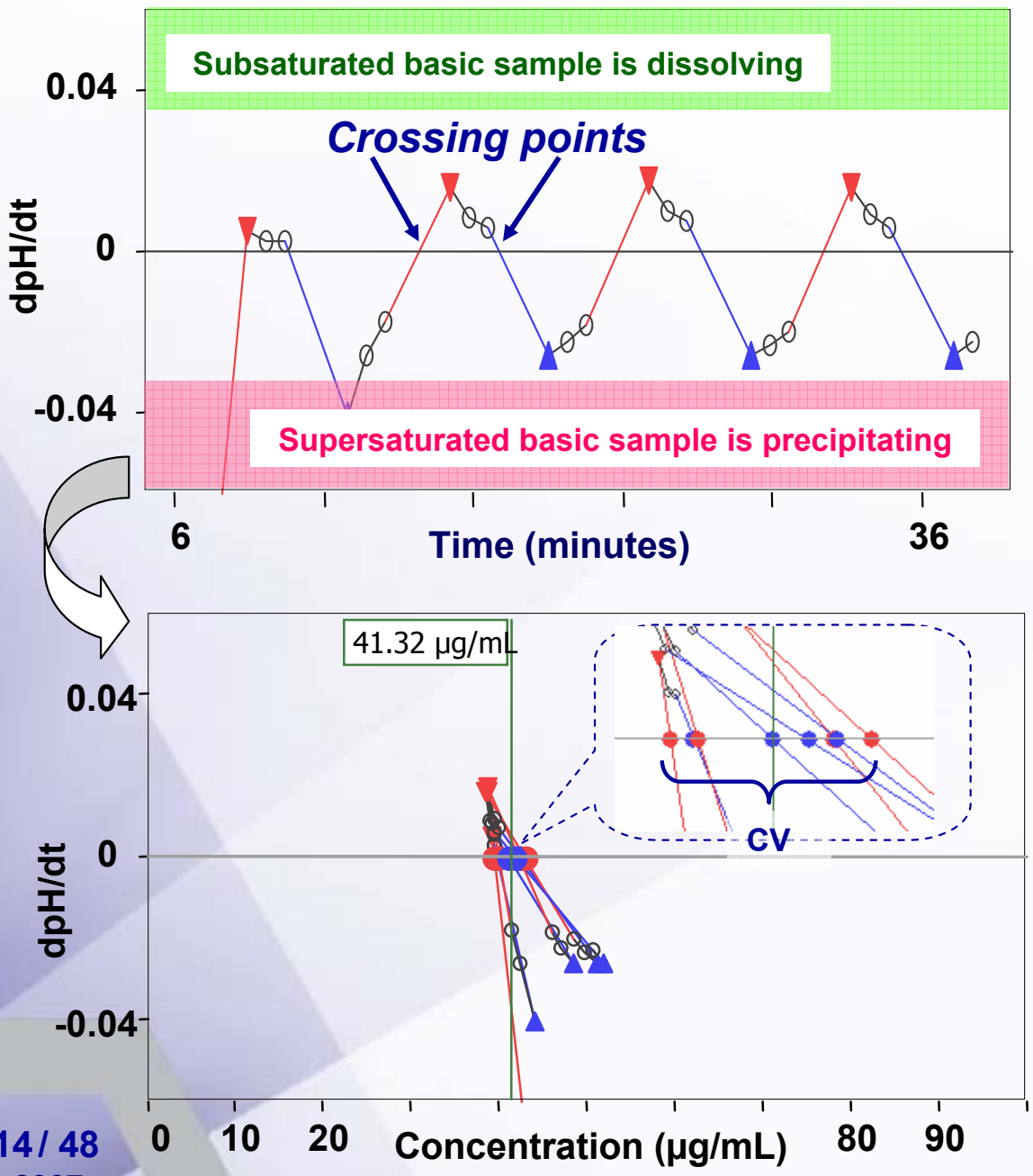


Black lines and circles - nothing added
Blue lines and triangles - KOH added
Red lines and triangles - HCl added

The system would be at equilibrium at the crossing points

- Chasing Equilibrium continues until a graph like this can be drawn. In the graph above, there are eight *crossing points*.
- After collecting a specified number of crossing points, the instrument adjusts pH back to the starting pH to re-dissolve the sample, then cleans the probes for the next experiment.

Calculating the result for Pindolol



- The concentration of unionized species at each point in the Crossing Point Graph is calculated. This requires
  - **weight of sample**
  - **total volume of solution**
  - **concentrations and volumes of acid and base dispensed**
  - **pH at each point**
  - **pK_a(s) of sample, both value and type (acid, base)***
- Gradient vs. concentration is plotted in the graph below
- The average value of all *crossing points* is the concentration of the unionized species at equilibrium.
- This is the Intrinsic Solubility
- CV shows the quality of assay

*** The procedure is sensitive to errors in pK_a – an error of 1 pK_a causes an error of 1 logS unit**

Pindolol is a chaser

- Pindolol is a chaser because its kinetic solubility is significantly higher than its Intrinsic solubility
- The neutral species of Pindolol forms a supersaturated aqueous solution
- It precipitates slowly, and would take a long time for all the substance to precipitate at a given pH

Everything a “Chaser”?

- When we initially discovered CheqSol – we thought every drug would supersaturate to some degree, and therefore “Chase Equilibrium”

- Our first paper presents 6 chasers:

Stuart, M. Box, K. Chasing equilibrium: measuring the intrinsic solubility of weak acids and bases. Anal. Chem. 2005 (77(4)) pp 983-990

Solubility of six compounds from our first paper

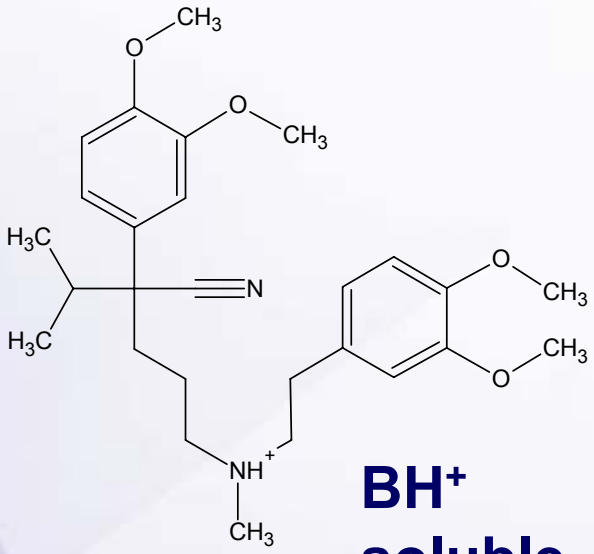
	pK_a	Sample weight (mg)	Time taken (min)	CheqSol		Literature
				Kinetic solubility (µg/mL)	**Intrinsic solubility (µg/mL)**	Intrinsic solubility (µg/mL)
Diclofenac	3.99	3.4-24	33	45 ± 6	**0.9 ± 0.1**	0.8 ± 0.2 [1]
Ibuprofen	4.35	6.2-51	43	180 ± 10	**50 ± 4**	49 ± 2 [1]
Warfarin	4.94	10-12	60	120 ± 1	**5.3 ± 0.2**	5.6 ± 0.3 [2]
Lidocaine	7.95	96-280	79	4600 ± 900	**3500 ± 100**	3810 ± 20 [3]
Propranolol	9.54	10-19	60	340 ± 20	**81 ± 6**	70 ± 20 [1]
Famotidine	6.77, 11.01	102-123	61	5900 ± 650	**740 ± 40**	1100 ± 200 [1]

- All measurements at 25ºC in aqueous 0.15M KCl solution
- Results for Propranolol and Famotidine are the mean of 6 (others are mean of 10)
- Time taken includes dissolution time

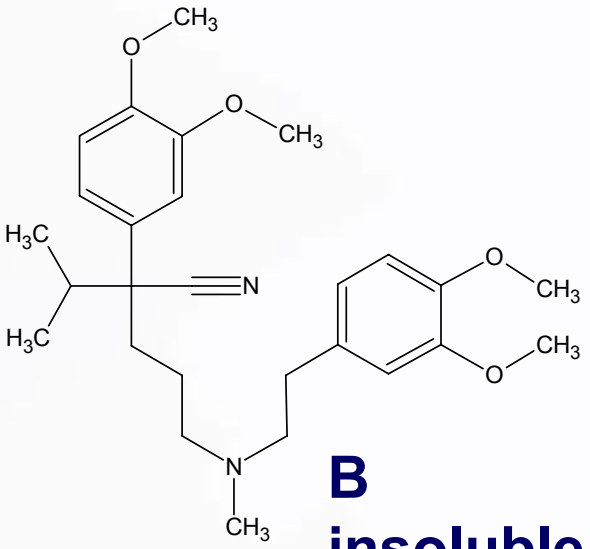
[1] Avdeef, A. Berger, C M. Brownell, C. Pharm. Res. 2000, 17 (10, 85-89
[2] Bergström, C A S. Strafford, M. Lazorova, L. Avdeef, A. Luthman, K. Artusson, P. J. Med. Chem. 2003, 46, 558-570
[3] Powell, M F. in Analytical Profiles of Drug Substances: Florey, K (ed); Academic \Press, San Diego 1986, 15, 761-779

Everything a “Chaser”?

- We then discovered some compounds that did not follow the “Chasing Equilibrium” process.
- We named these compounds “Non-Chasers”



BH^+ soluble

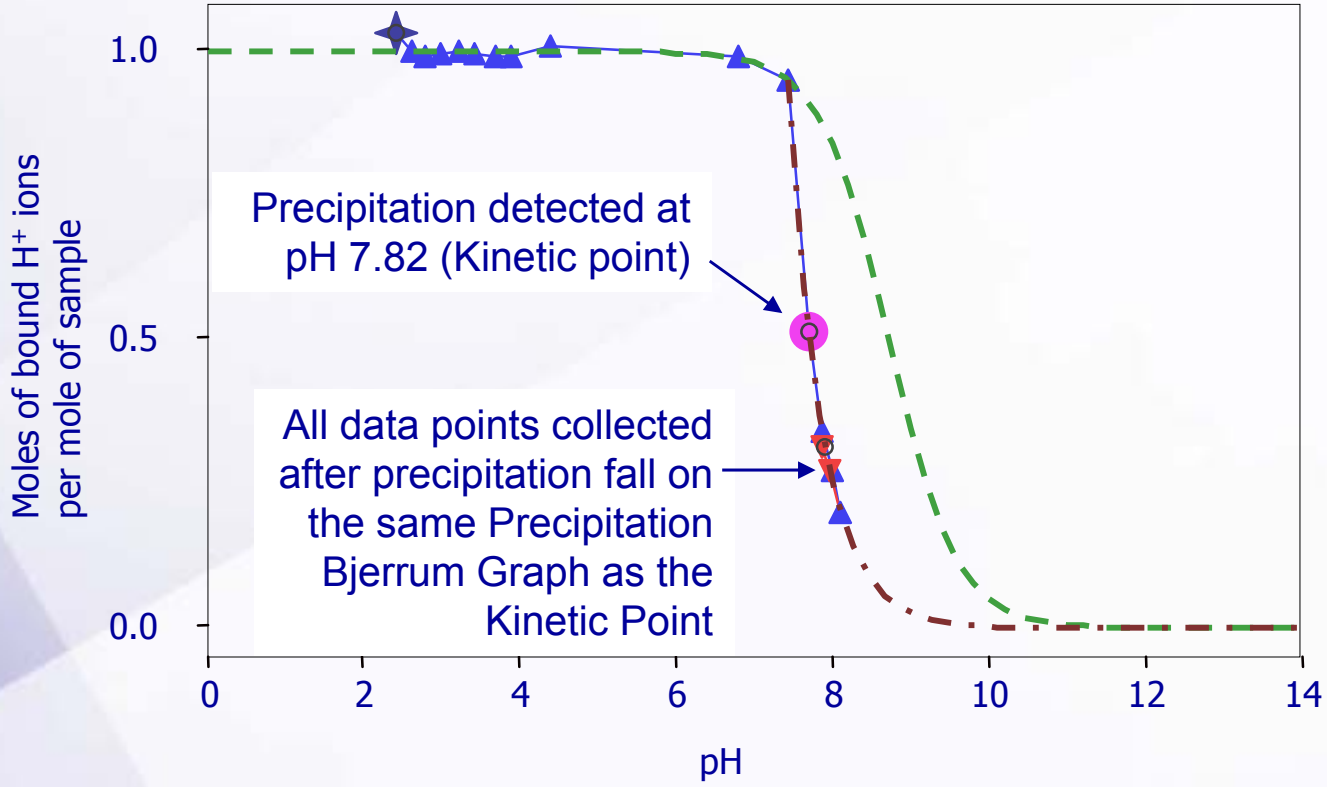


B insoluble

- Example – Verapamil (tertiary amine – a base with pK_a of 8.72 @25°C, 0.15M ionic strength)

Verapamil Bjerrum Curve

- For Verapamil, all points collected, including the kinetic solubility, fall on the Precipitation Bjerrum Graph
- After titrating with base, the instrument adds acid to check that the points are still on the Precipitation Bjerrum Graph. If they are, then …
- Kinetic solubility = Intrinsic solubility = the solubility value required to fit a precipitation curve to the data points
- Assay took just 19 minutes to measure kinetic and Intrinsic solubility

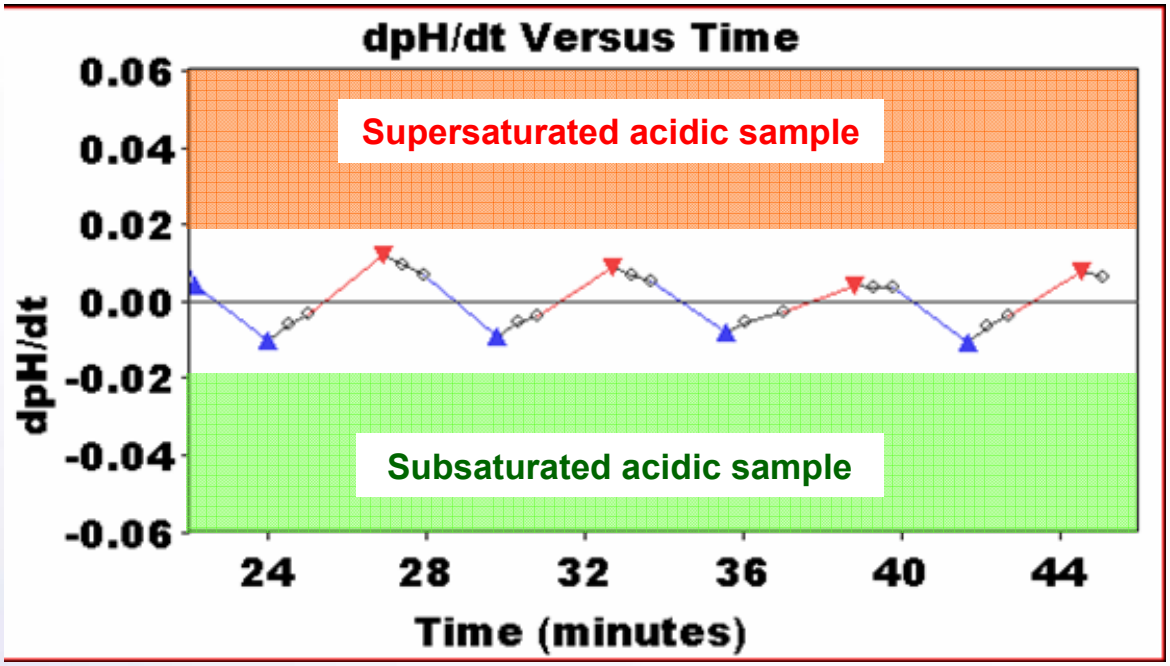


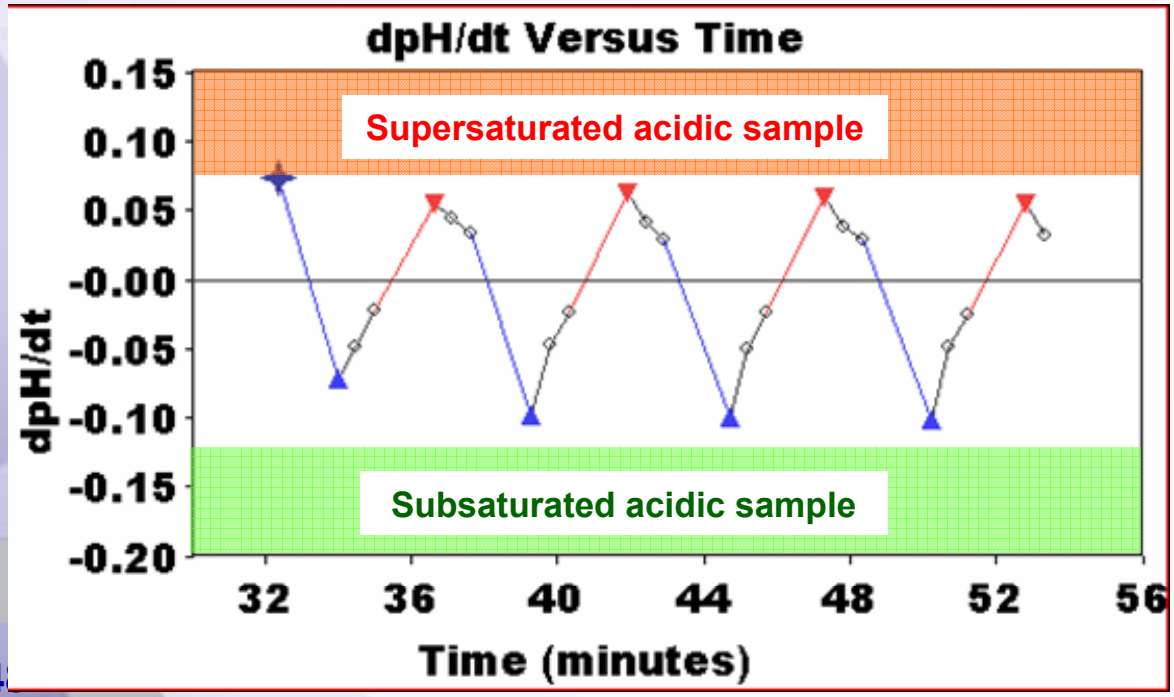
Other Non-Chasers

- Chlorpromazine, Imipramine, Quinine, Amitryptyline, Diphenhydramine, Nortriptyline, Desipramine, Diltiazem, Deprenyl.

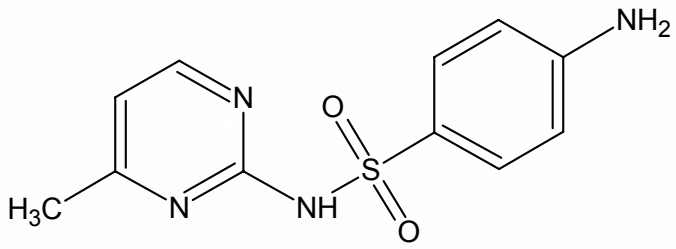
- Questions raised:
  - What determines the degree to which a compound will supersaturate or not?
  - Can we predict supersaturation behaviour from structure?
  - Do chasers precipitate and dissolve at equal rates?

Investigating pH change with time





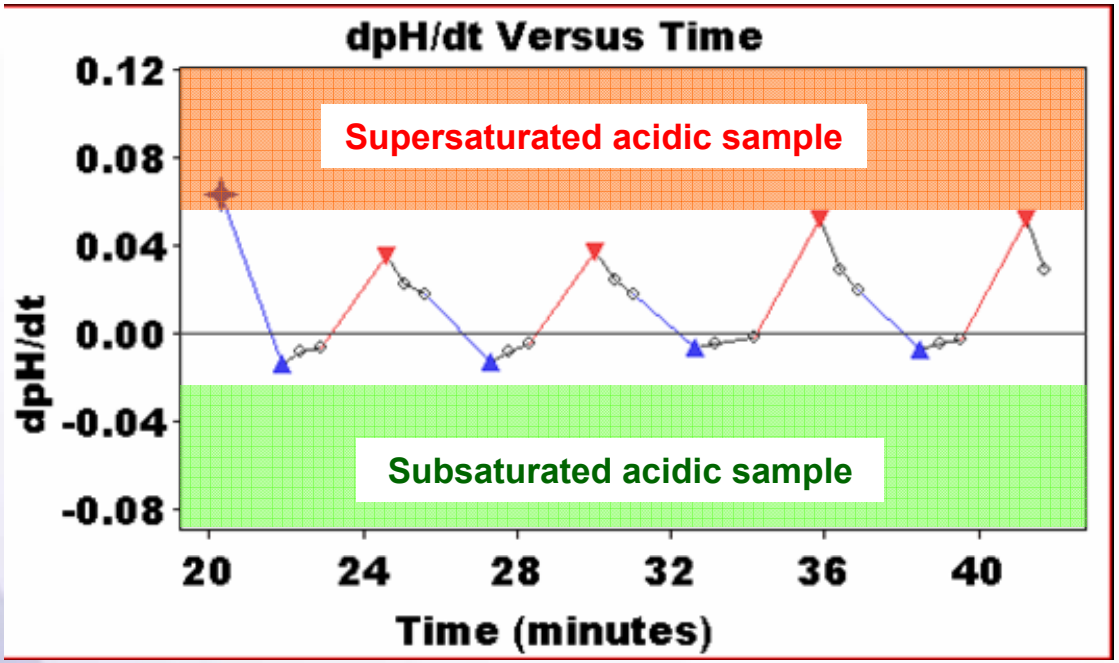
Sulfamerazine

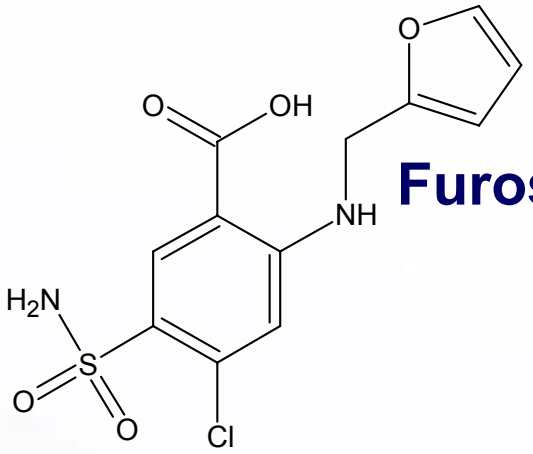


Most of the early compounds we investigated show a tight symmetry.

Piroxicam

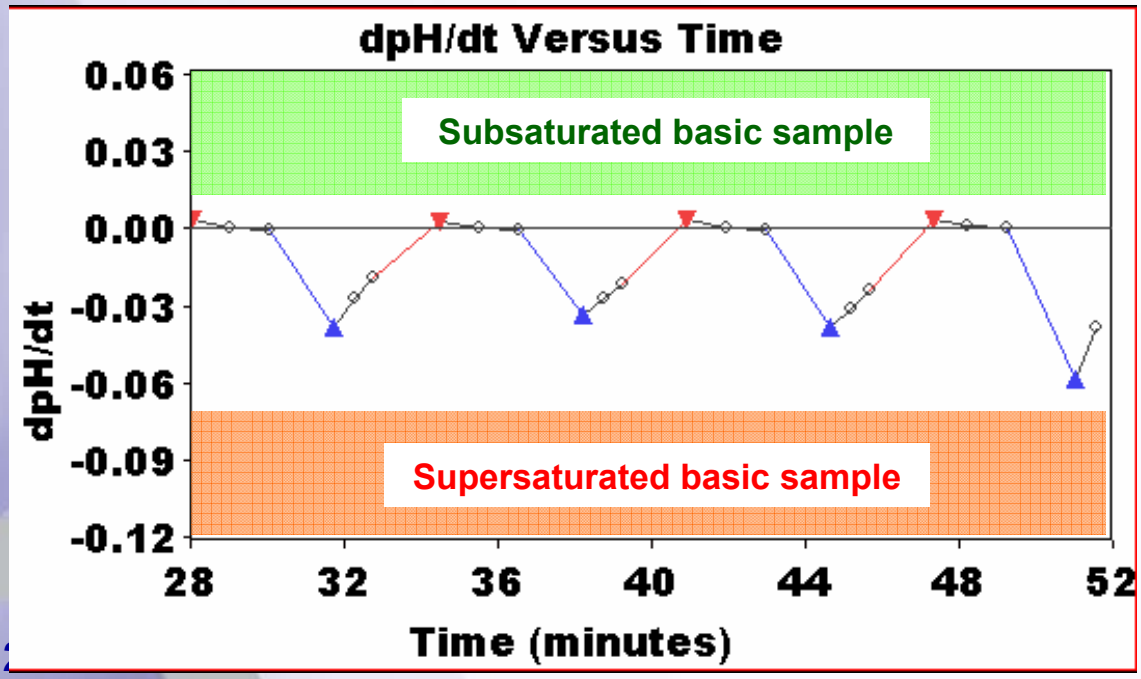
Investigating pH change with time

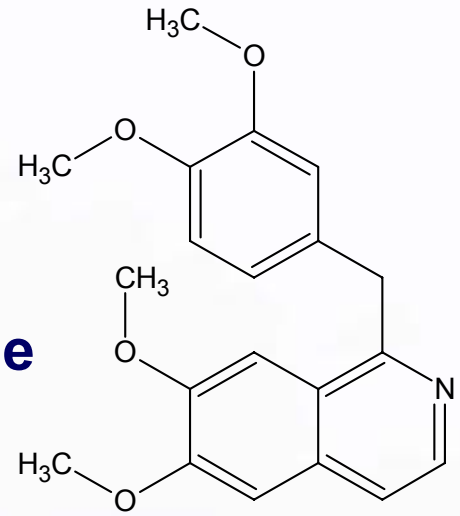




Furosemide

Some compounds show a clear offset!





Papaverine

A new tool: “Precipitation Rate Graph”

These novel graphs enable precipitation rates and dissolution rates to be estimated when precipitates are close to equilibrium.

$$\text{Precipitation rate} = \frac{dC}{dt} = \frac{-\frac{dpH}{dt} \times BI}{\text{Average molecular charge}}$$

BI = Buffer Index

C = Concentration

Precipitation rate is plotted on the Y axis. Concentration is plotted on the X-axis.

The concentration where the graphs intersect the X-axis is equivalent to the intrinsic solubility.

A new tool: “Precipitation Rate Graph”

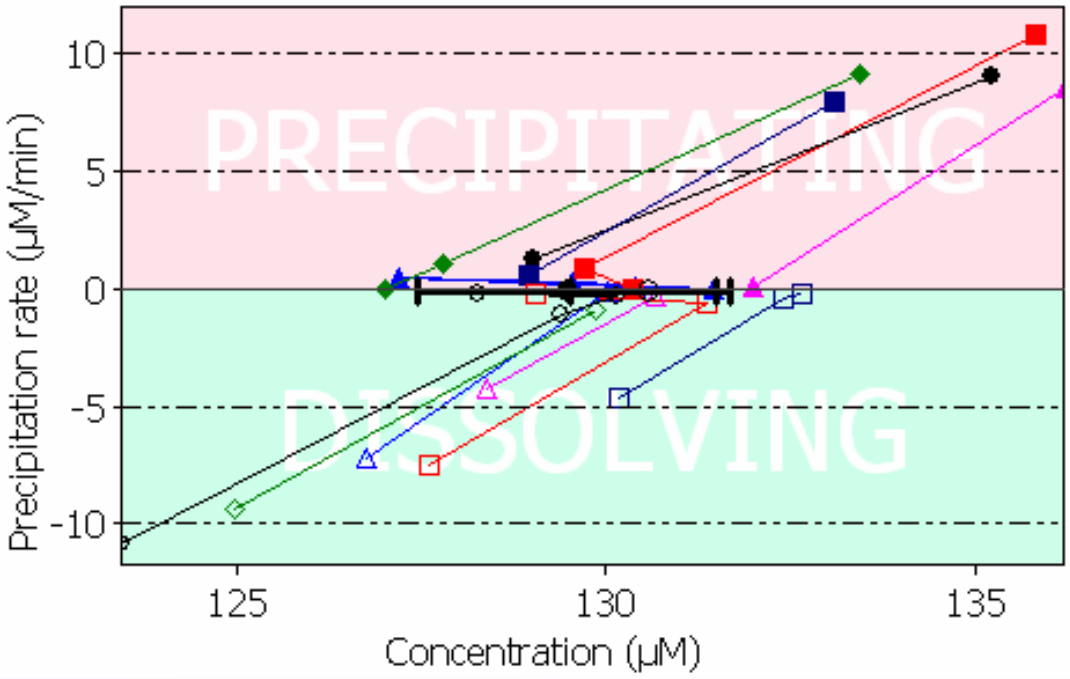
Example: **Ibuprofen** (Acidic Chaser)

Negative rate of precipitation = sample dissolving.

Filled circle ● = solubility determined from the precipitating sample..

Open circle ○ = solubility determined from the dissolving sample.

The intrinsic solubility reported by CheqSol is equivalent to the mean of the intercepts at Y = 0



The Four Class Model

Using the Precipitation Rate graph to investigate ~100 ionisable drugs, we have found that there appears to be four classes of behaviour.

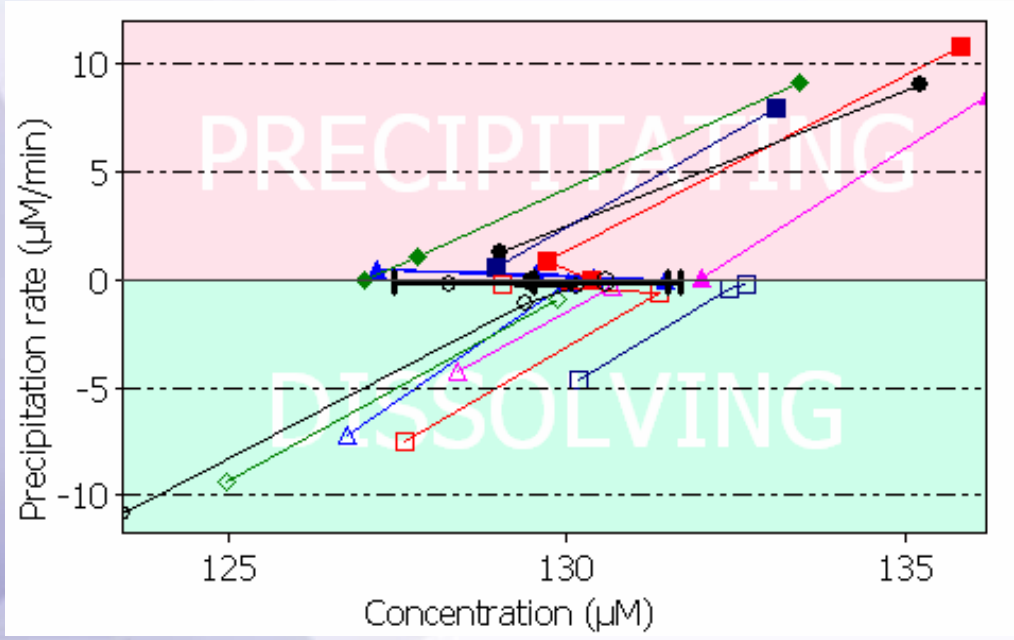
	Slow Precipitator	Fast Precipitator
Slow Dissolver	**"Chasers"** Full chaser for both precipitation and dissolution *Examples:* Ibuprofen, Benzocaine, Benzthiazide.	**"Non-Chasers"** No precipitation chase but can chase while dissolving *Examples:* Nortriptyline, Amitryptyline, Imipramine.
Fast Dissolver	**"Super Chasers"** Chases during precipitation but not while dissolving *Examples:* Tolmetin, Papaverine, Chlorzoxazone.	**"Ghosts"** No chasing possible for either precipitation or dissolution *Examples:* None yet discovered.

Precipitation Rate Graph: Chaser

Molecules that precipitate slowly from supersaturated solutions, and also dissolve slowly from crystalline solids.

Well over half the marketed drugs we have measured at Sirius have been Chasers, which can be acids, bases or ampholytes.

Chasers are well-behaved; however, we find that many drugs in development are not full Chasers.

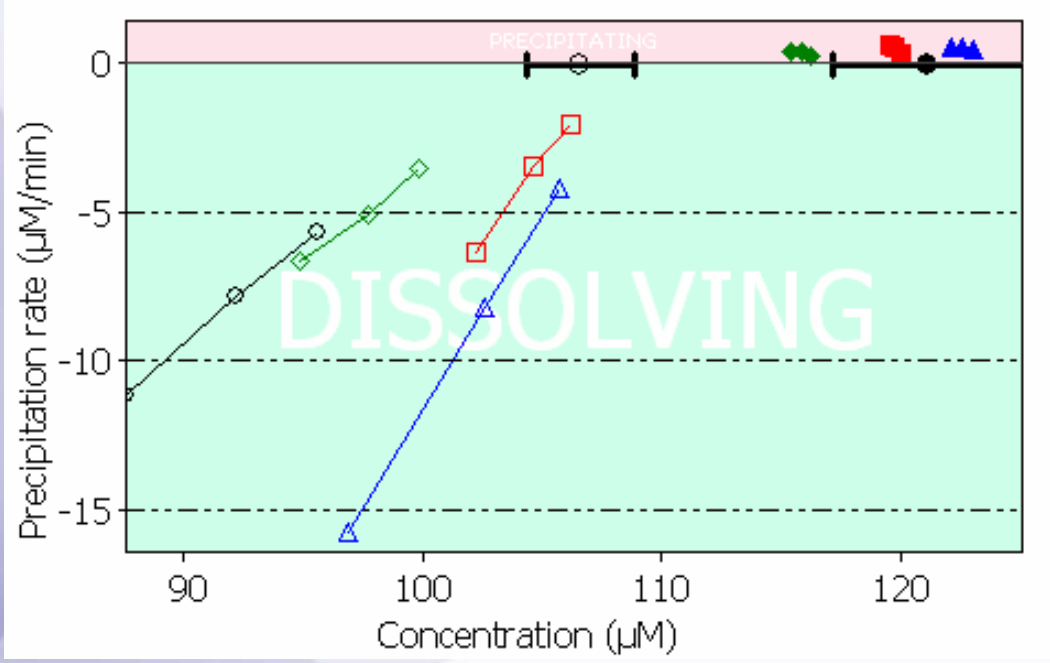


We can measure rates on both sides of the graph

Precipitation Rate Graph: Non Chaser

Molecules that precipitate very quickly without supersaturating, but dissolve slowly.

Some of the bases we have measured have been Non-Chasers. We very rarely see acid Non-Chasers.

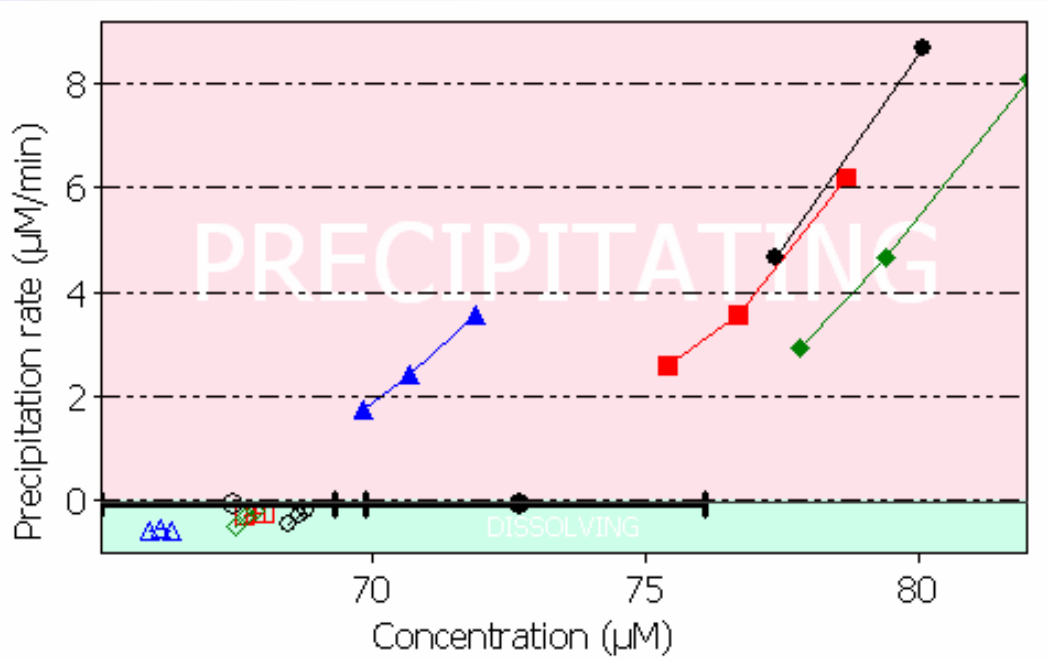


Precipitates so quickly, we can only collect good rate data on the dissolving part of the graph

Precipitation Rate Graph: Super-Chasers

Molecules that precipitate slowly and dissolve quickly.

We have seen only a few examples of super-chasers. The graph below shows data from an acid, Tolmetin. Other examples include Papaverine (a base) and Chlorzoxazone (an acid).



Dissolves so quickly, we can only collect good rate data on the precipitating part of the graph

Ghosts

Molecules that precipitate very quickly and dissolve very quickly.

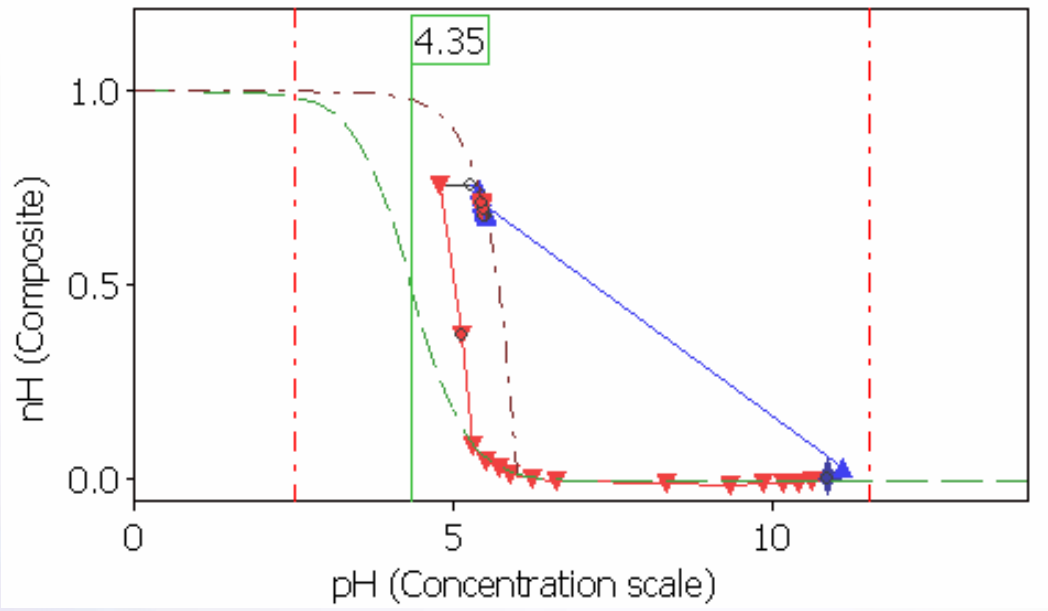
We postulate the existence of this class of molecule, but we have never seen a drug that shows this behaviour.

We would be able to measure its solubility by the same method we use to measure Non-Chasers.

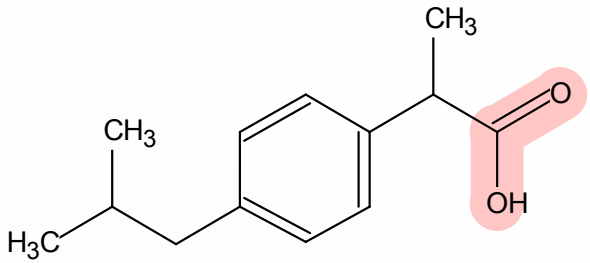
Data collection would be very fast, and all the data points would fall on the precipitation Bjerrum Curve, both for titration with KOH and with HCl.

Examples of drugs classified by the Four-Class model

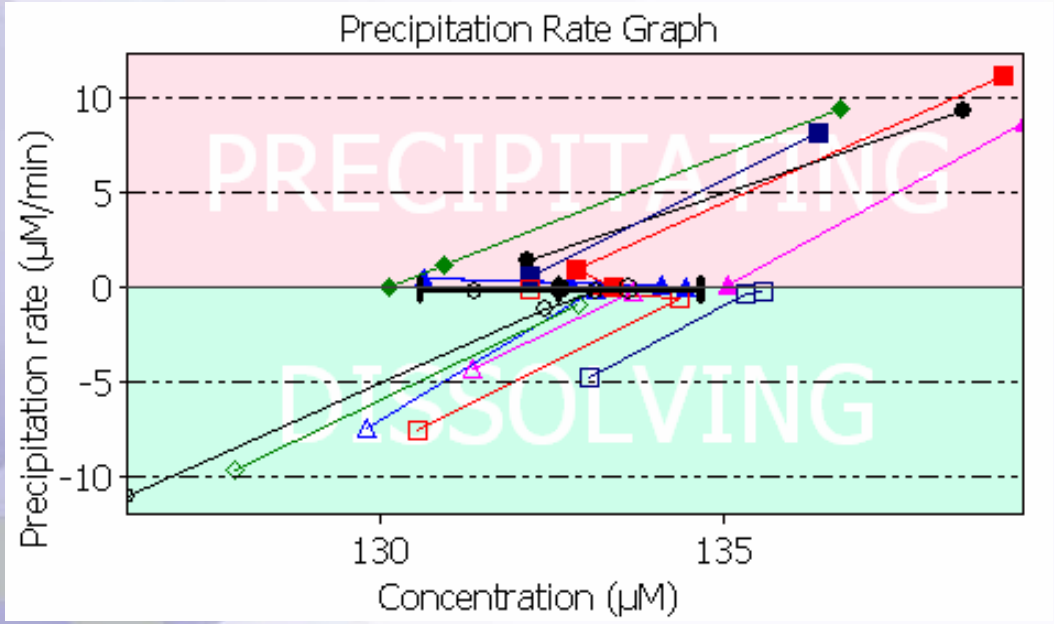
Chaser



Ibuprofen



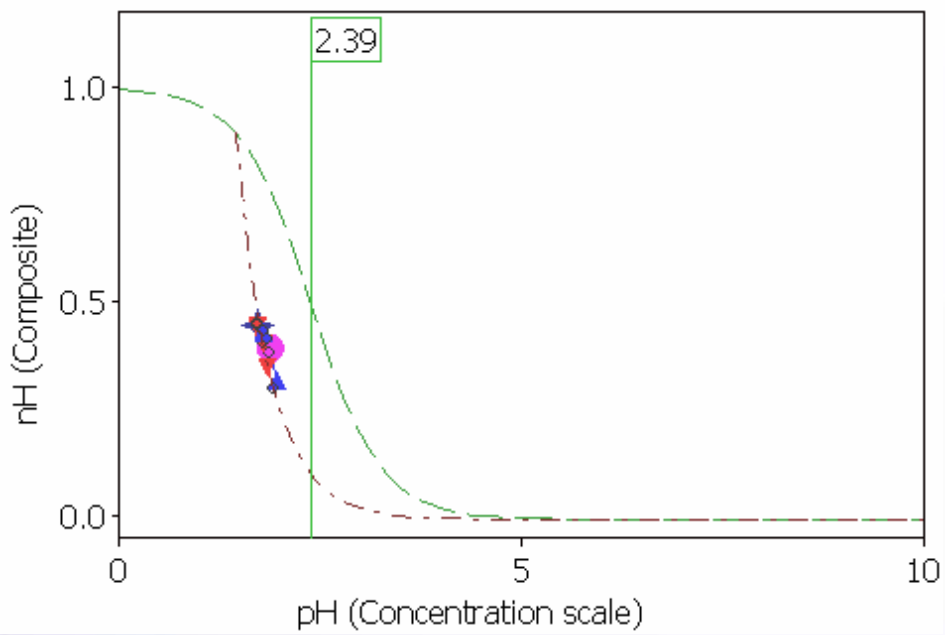
LogS = -3.61

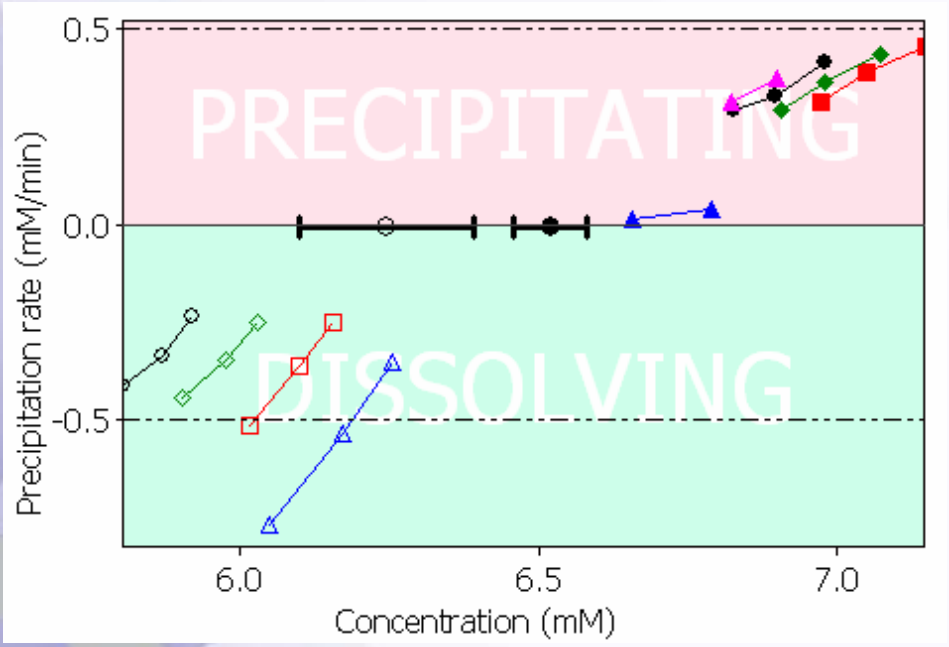


SLOW PRECIPITATING, SLOW DISSOLVING

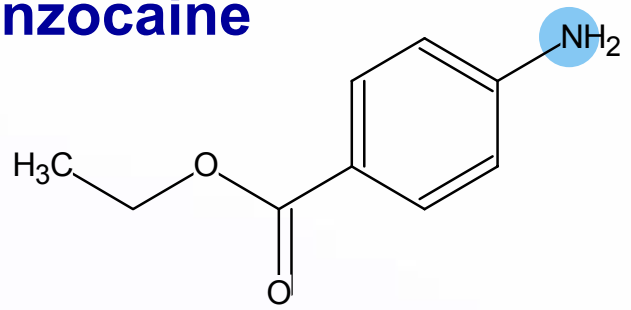
A classic Chaser

Chaser





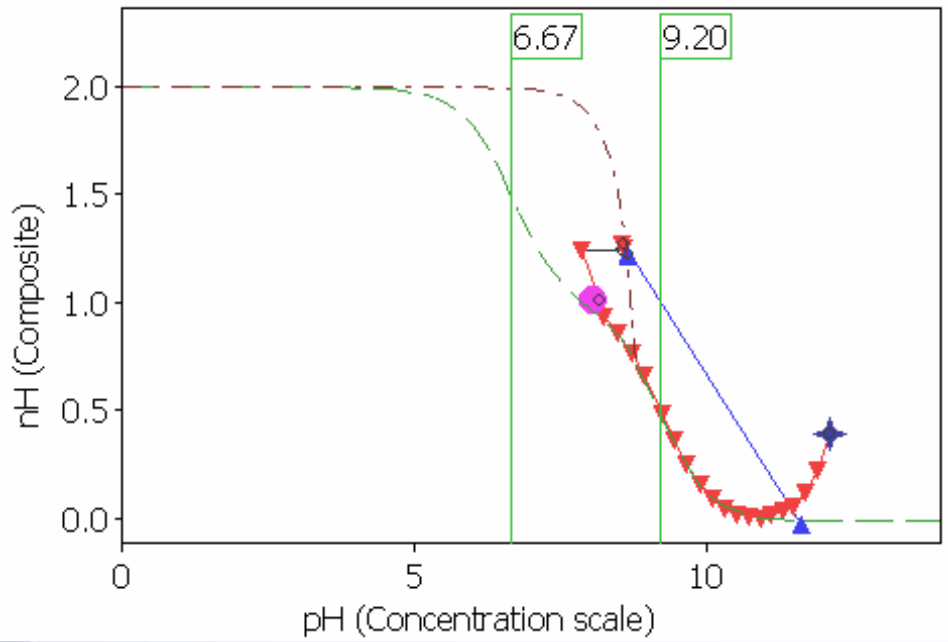
Benzocaine



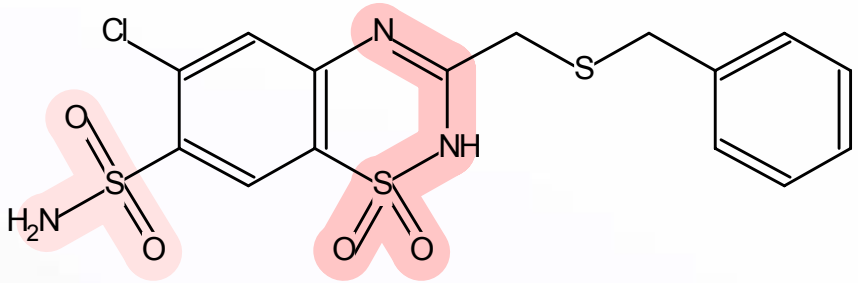
LogS = -2.19

Very high rates observed for this compound

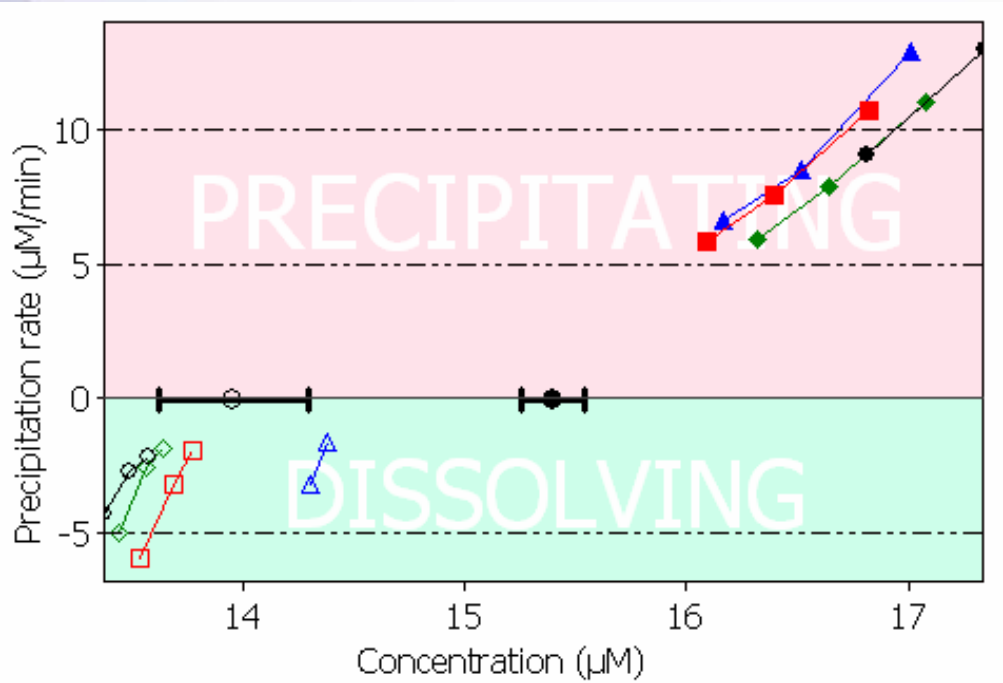
Chaser



Benzthiazide

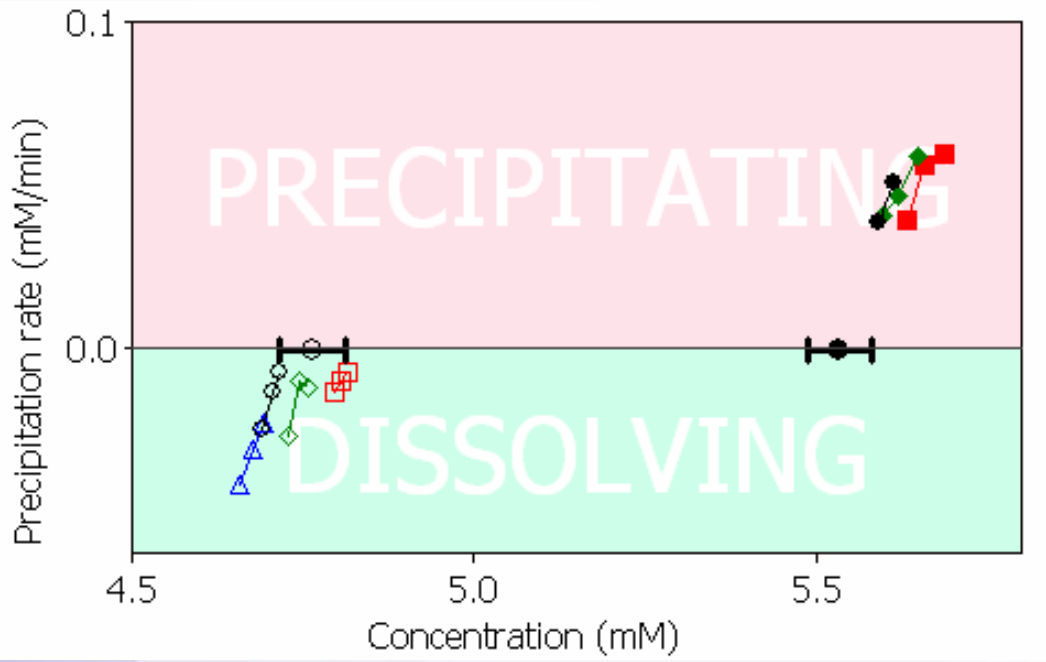


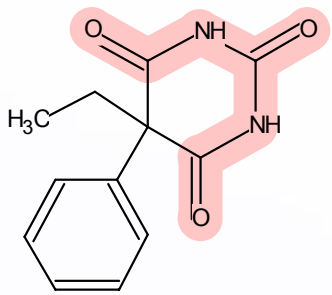
LogS = -4.84



Precipitating as one form, dissolving as another

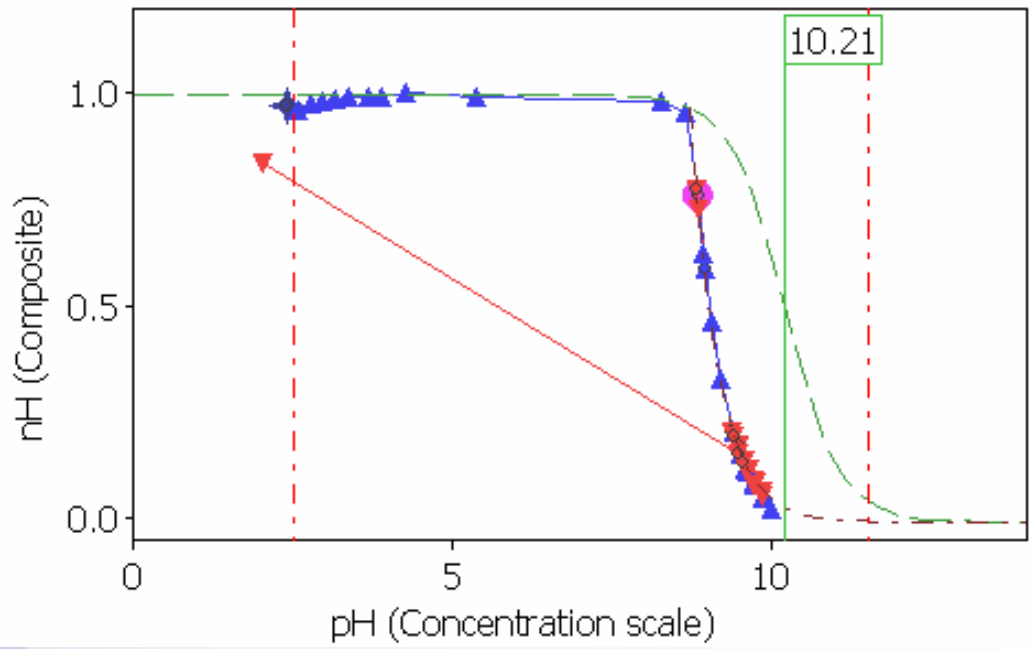
Precipitation rate graph for Phenobarbital.





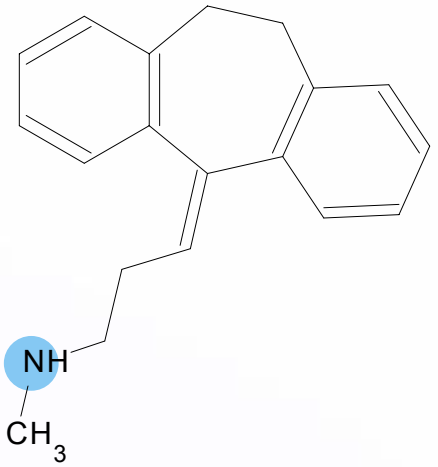
Large deviation between dissolution solubility and precipitation solubility implies that after it precipitates, Phenobarbital converts into a different form which is harder to dissolve and has a lower solubility.

Many drugs show similar behaviour, including Sulfamerazine, Ketoprofen, Benzthiazide, Thyroxine and Ciprofloxacin.



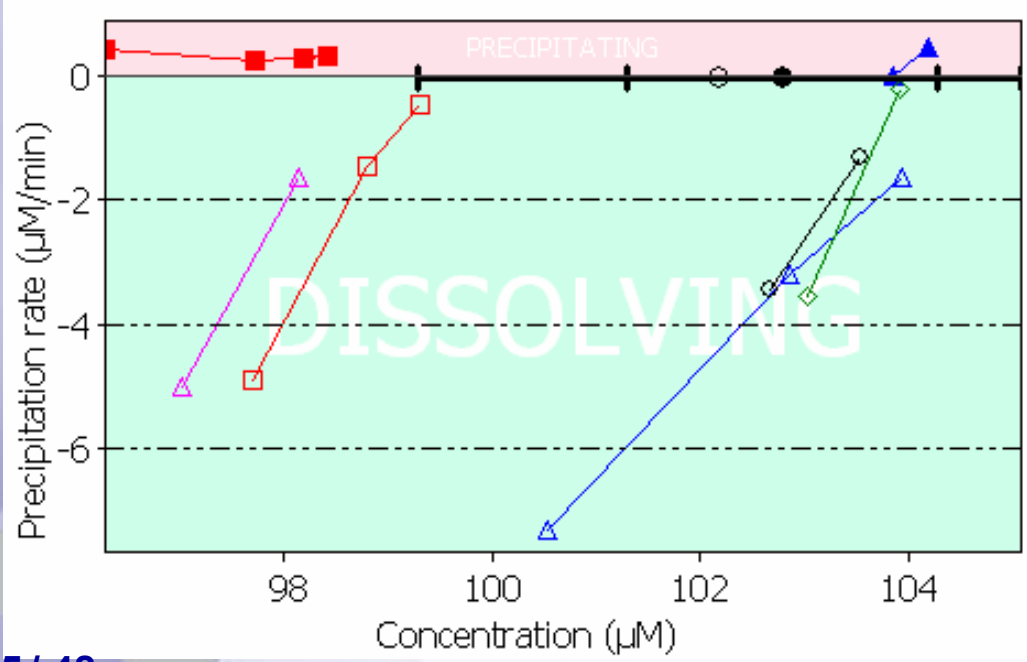
Non-chaser

Nortriptyline



LogS = -3.98

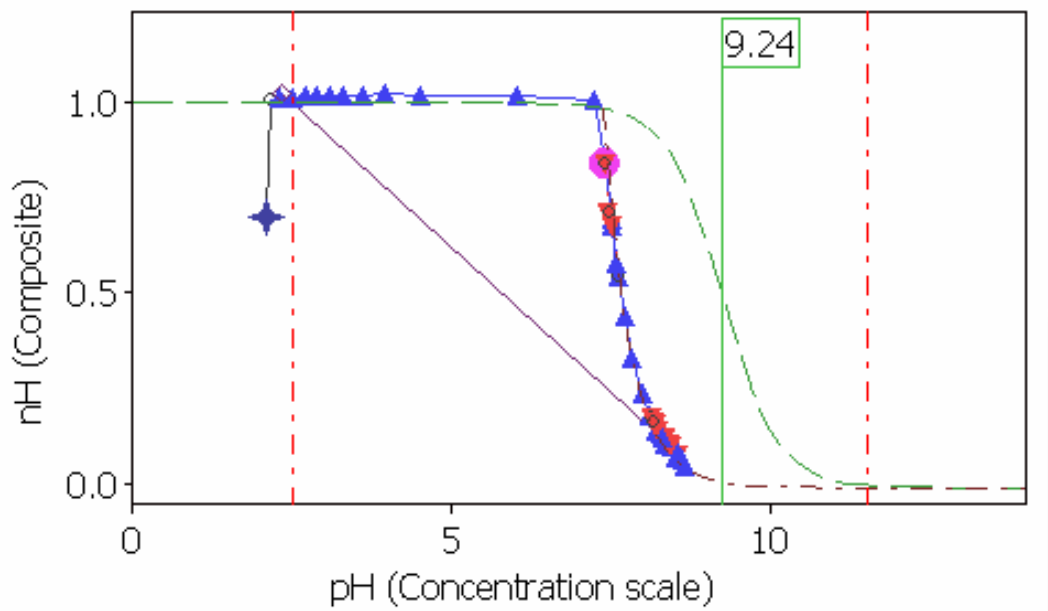
SLOW DISSOLVING, FAST PRECIPITATING



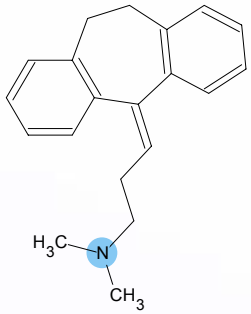
No data here, because it precipitates so fast that we can't collect good data using our standard "chasing" procedure

We still get the correct solubility result by curve-fitting

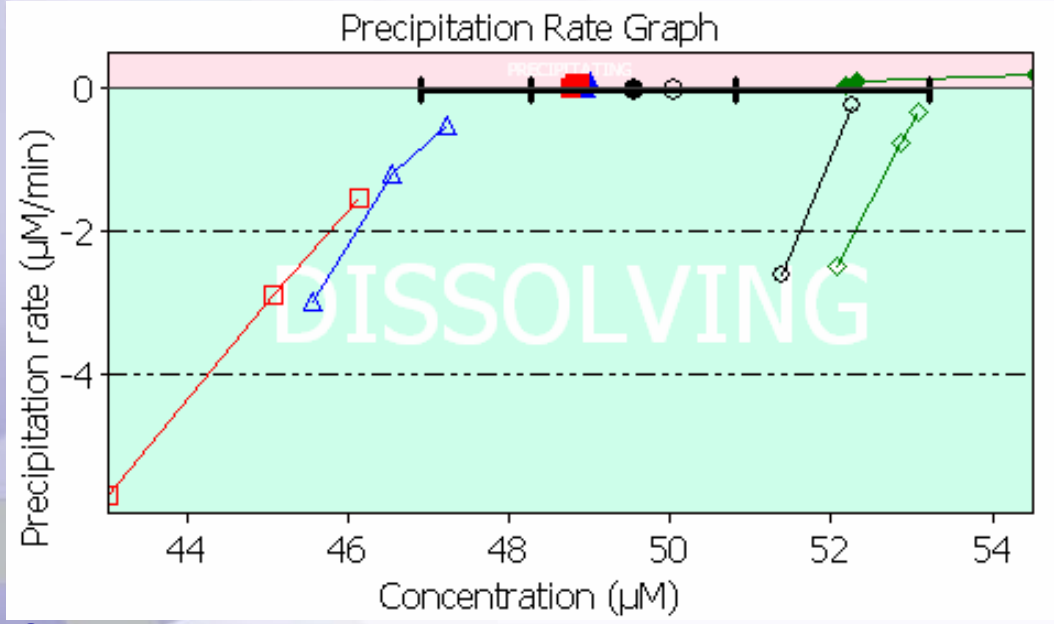
Non-chaser



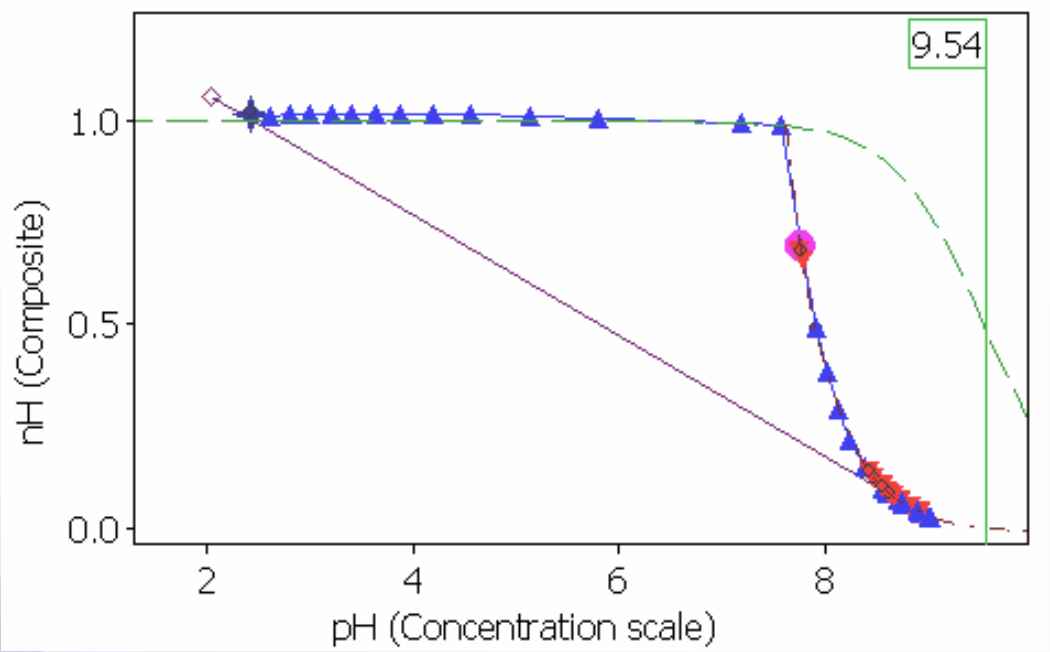
Amitriptyline



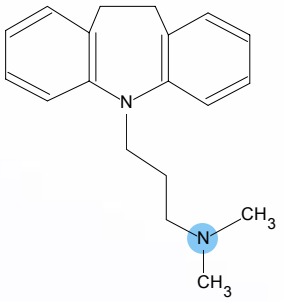
LogS = -4.39



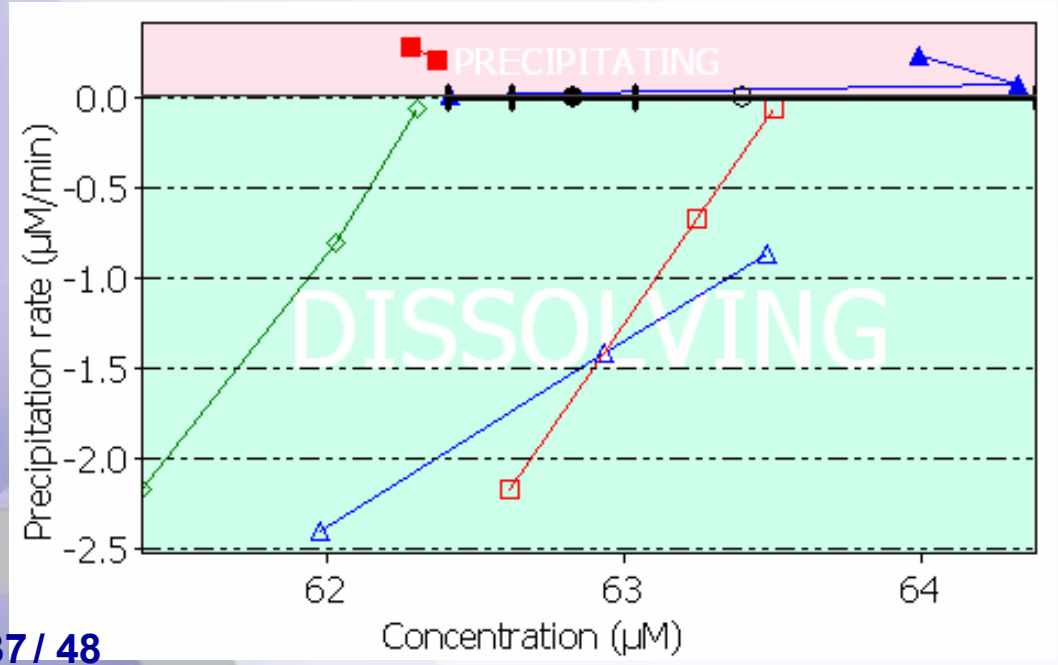
Non-chaser



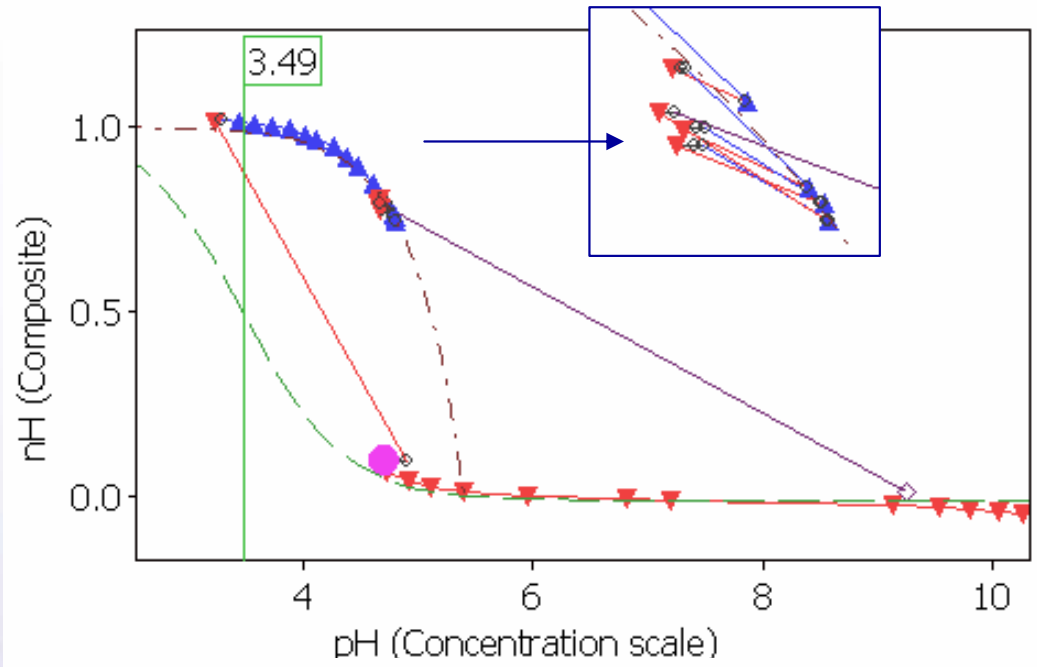
Imipramine



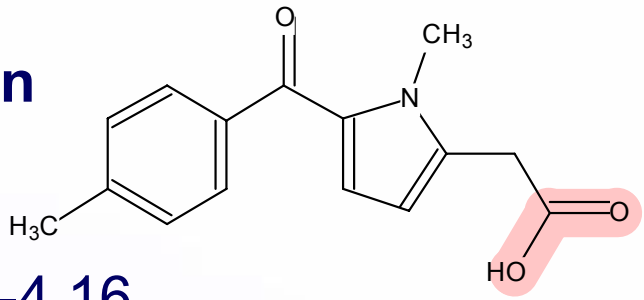
LogS = -4.20



Super Chaser

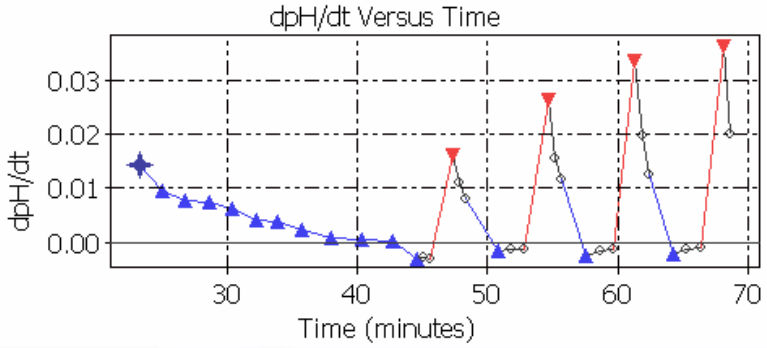


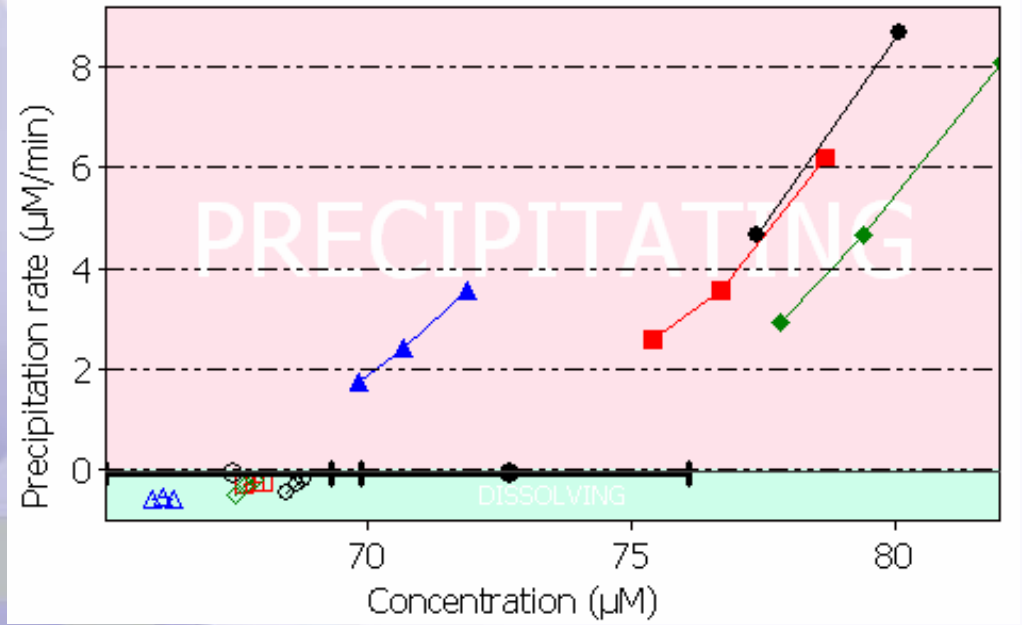
Tolmetin



LogS = -4.16

SLOW PRECIPITATING, FAST DISSOLVING

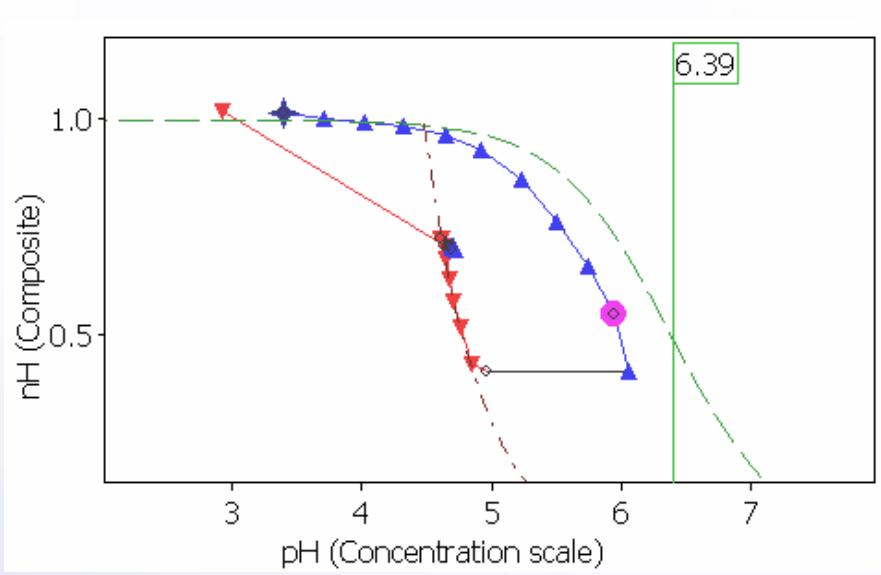




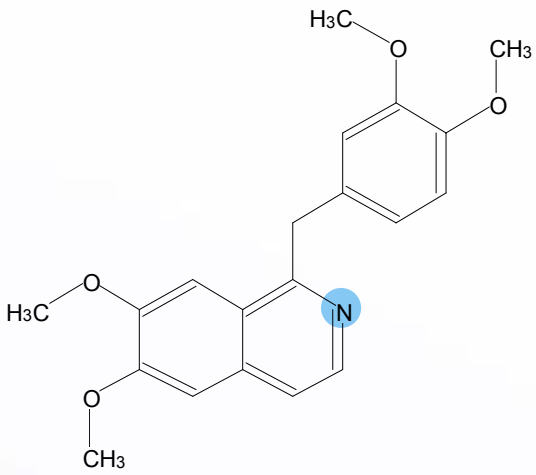
No data here, because it dissolves so fast that we can't collect data using our standard procedure

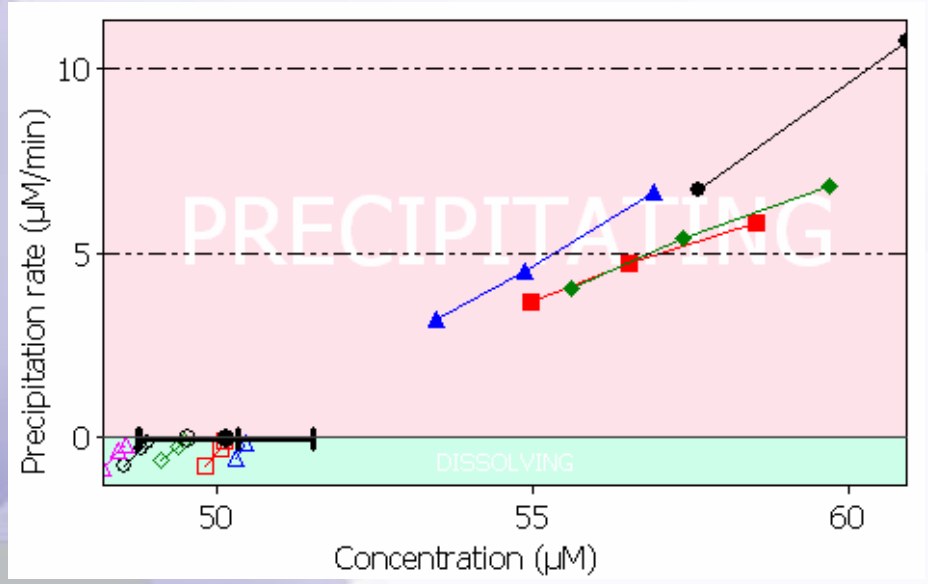
Super Chaser

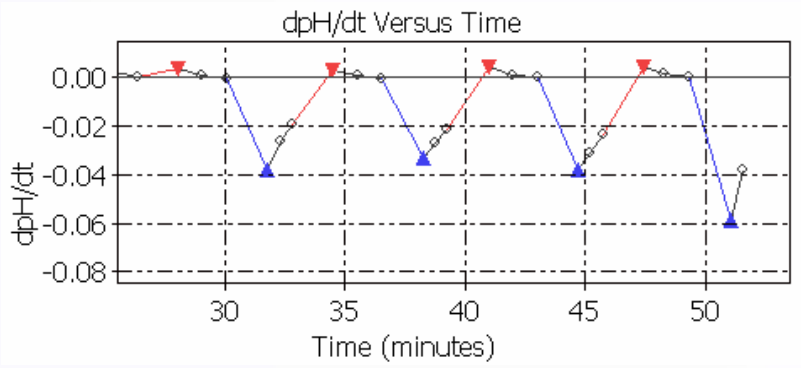
Papaverine



LogS = -4.30

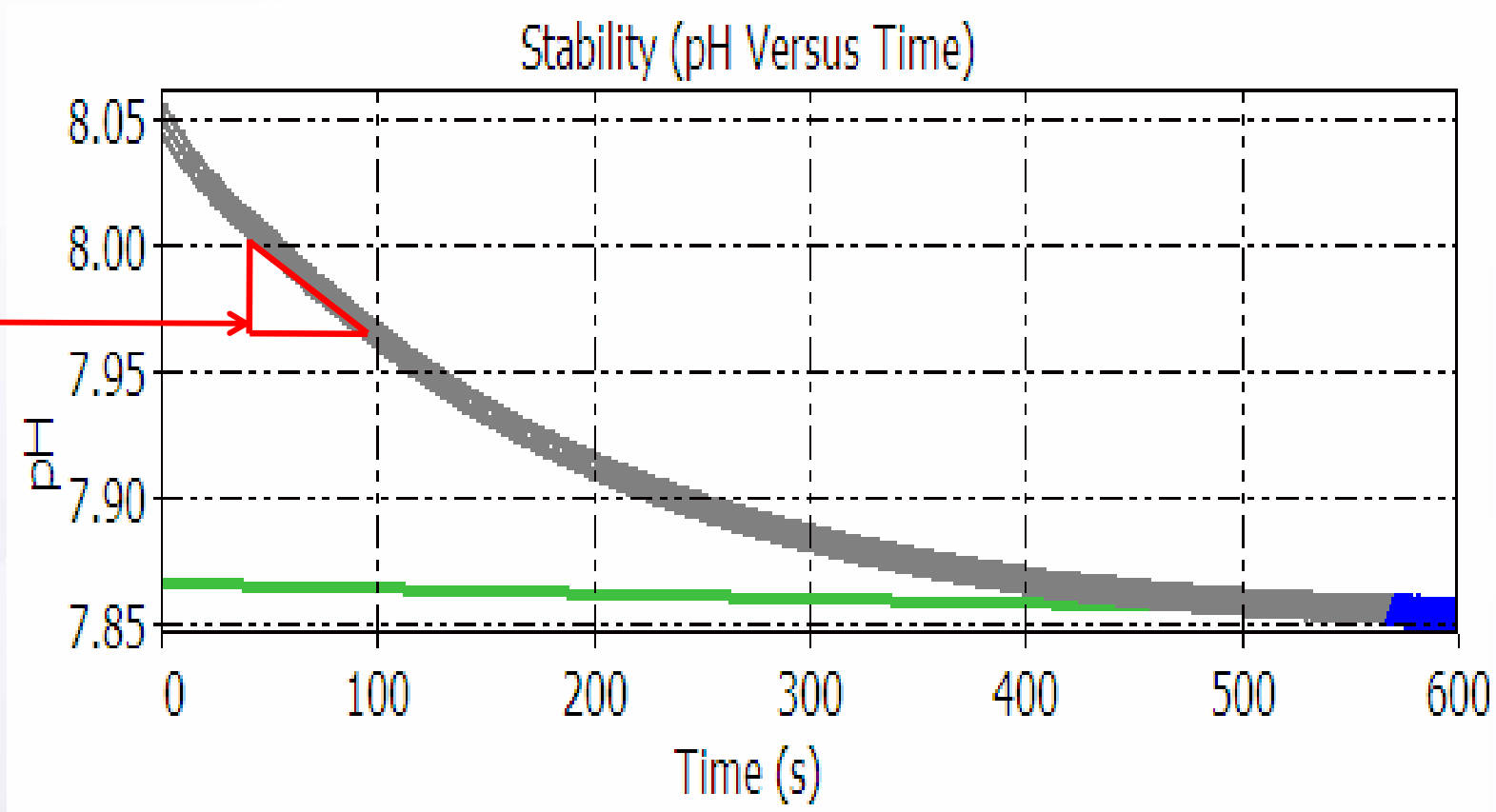






Chasers – Recording the Gradient

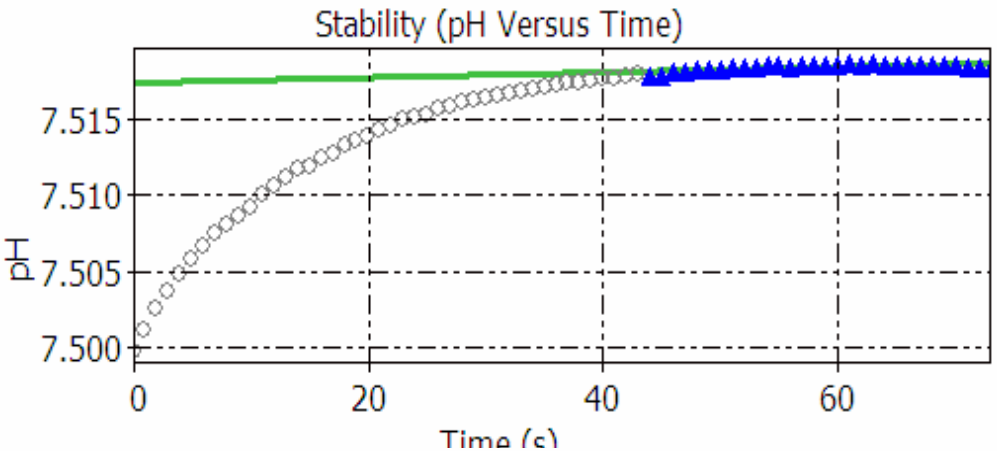
Typical gradient collection during "Chasing Equilibrium " method



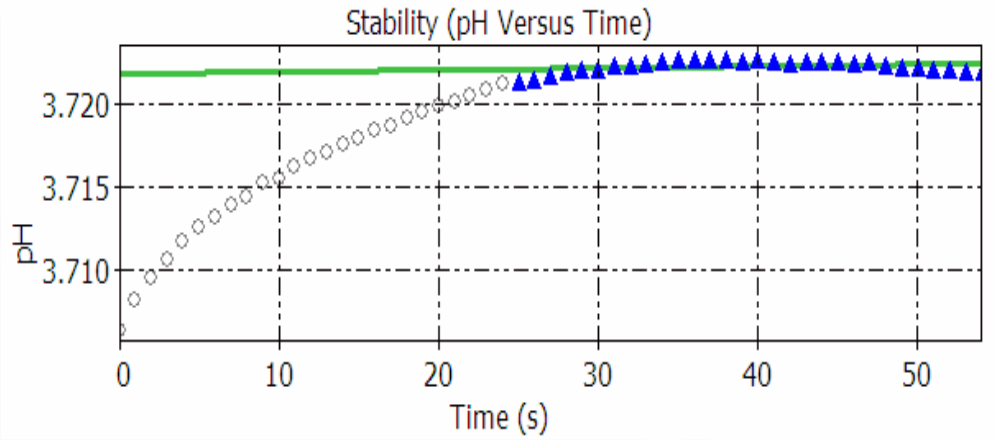
“Classic Method”

Real-time monitoring of the pH change to wait until equilibrium has been reached.

Method should work quickly for Non-Chasers (starting from dissolved)



Method should work quickly for “Super Chasers” (starting from solid)

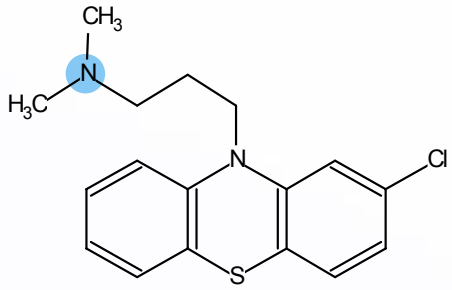


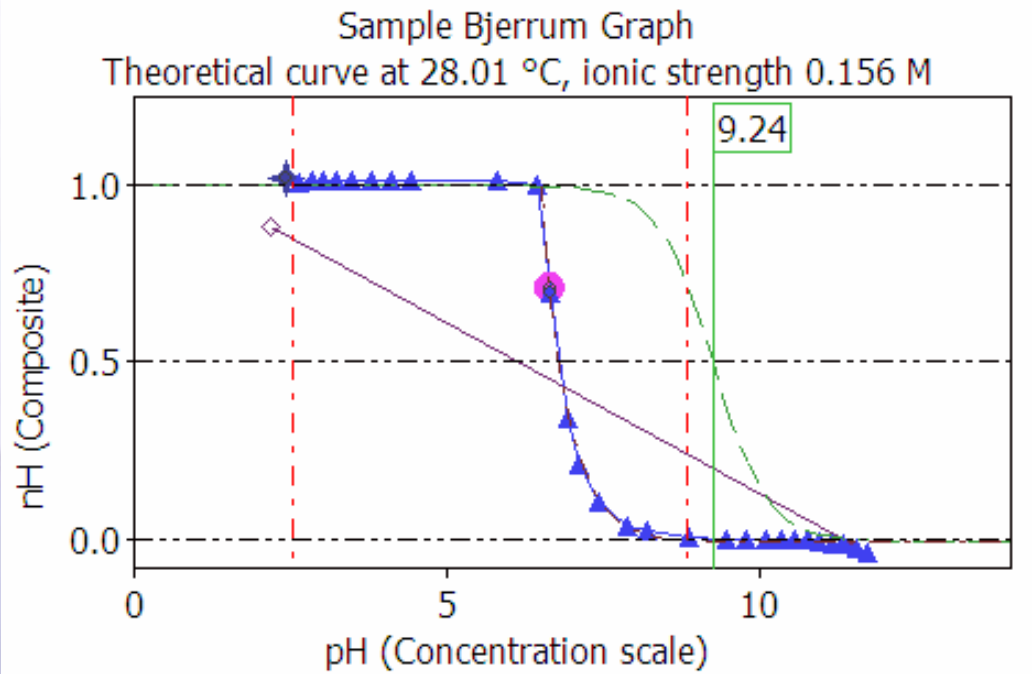
Method will be too slow for Chasers!
(in either direction of titration)

“Classic Method” – Non Chaser

Chlorpromazine

Starting from dissolved

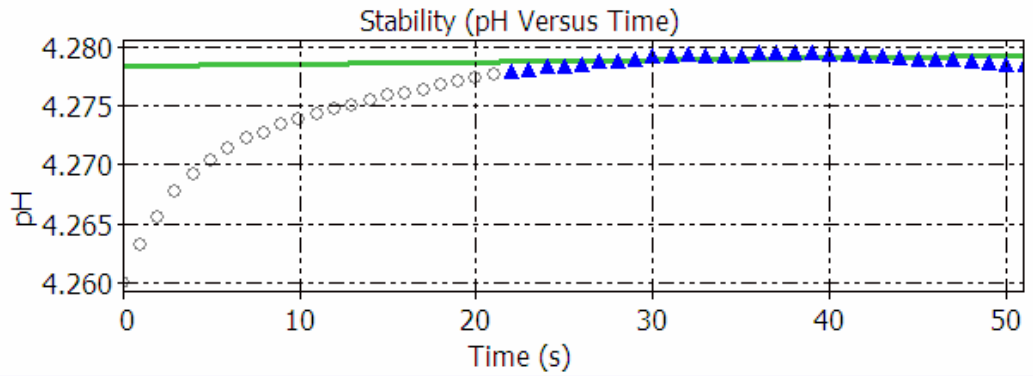




LogS = -5.09

Experiment time: 45mins

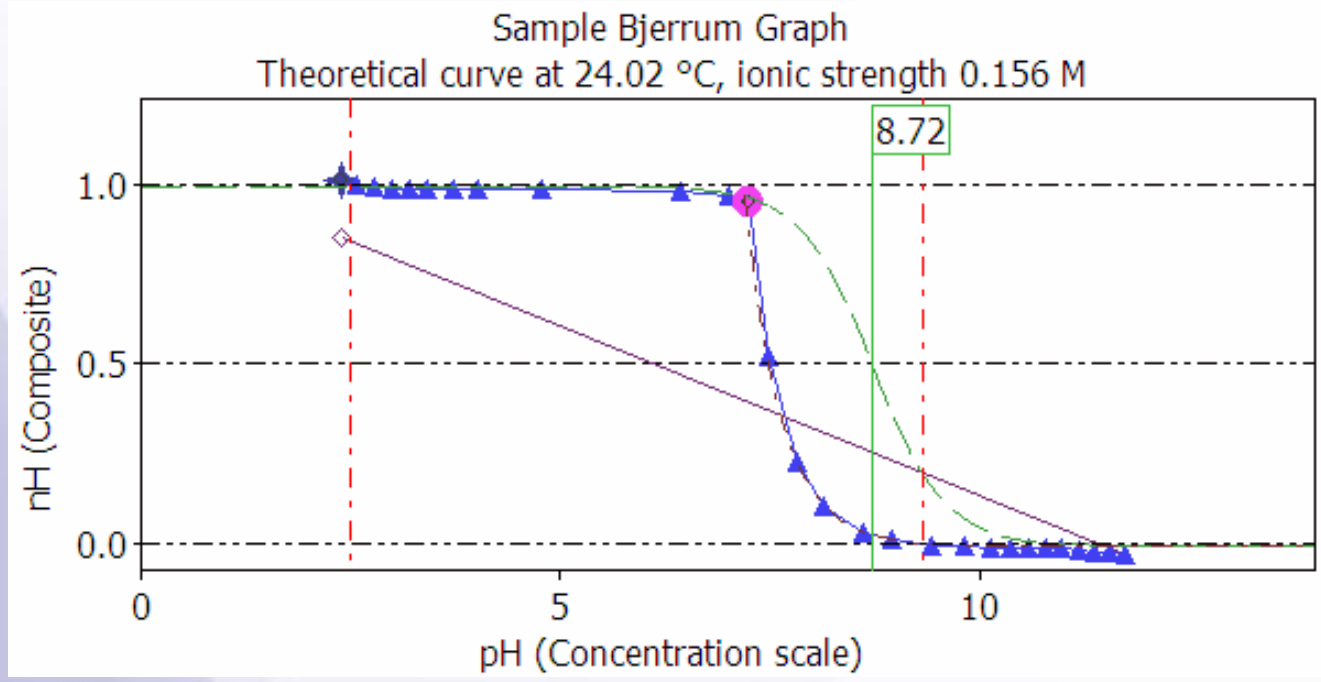
Average wait: ~ 50s

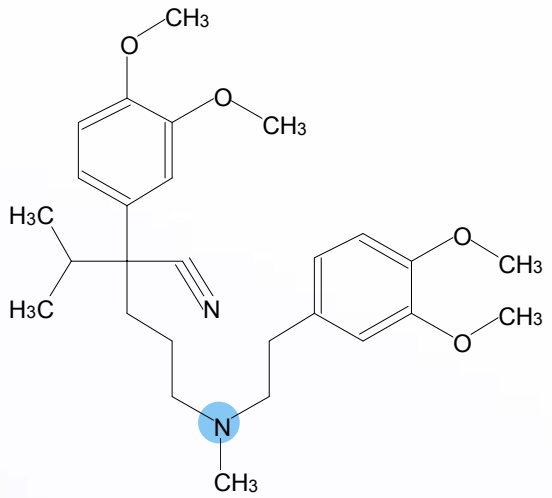


“Classic Method” – Non Chaser

Verapamil

Starting from dissolved

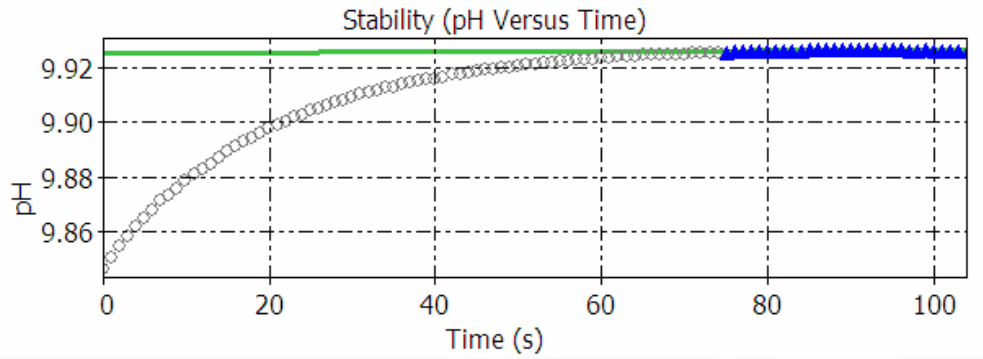




LogS = -3.95

Experiment time: 39mins

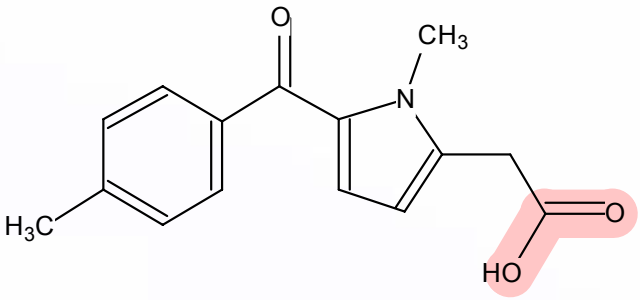
Average wait: ~110s

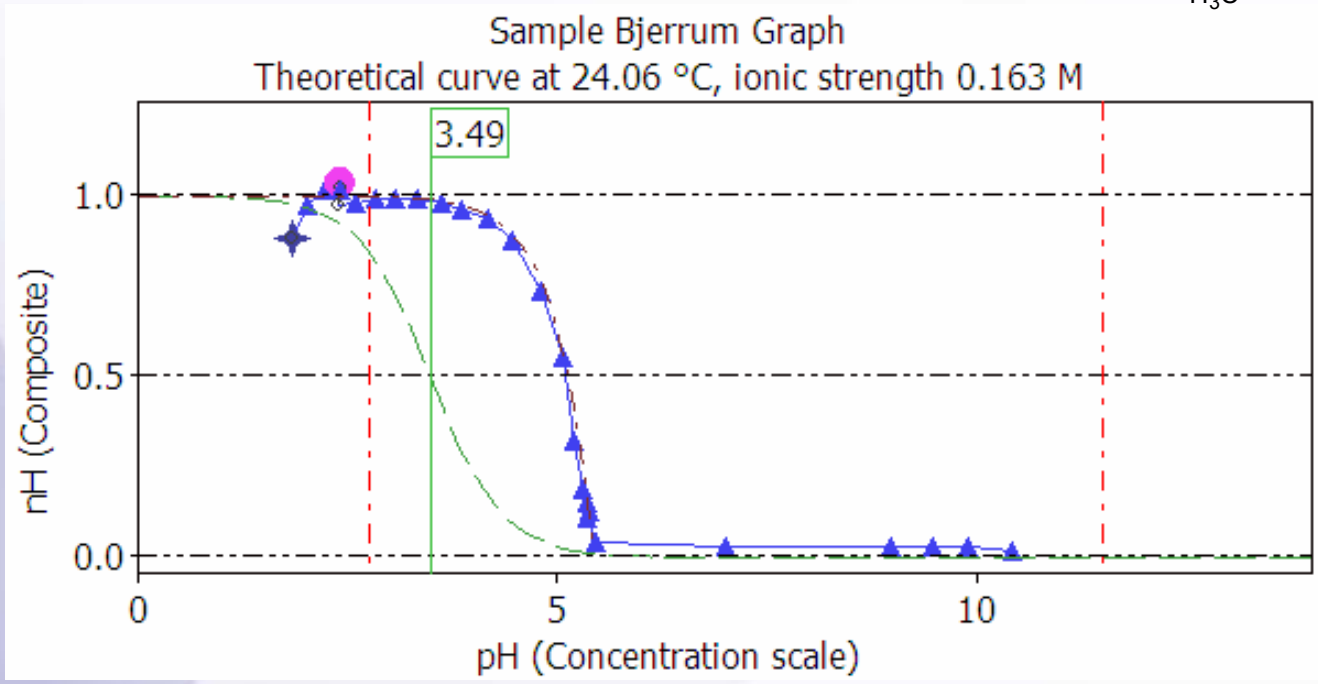


“Classic Method” – Super Chaser

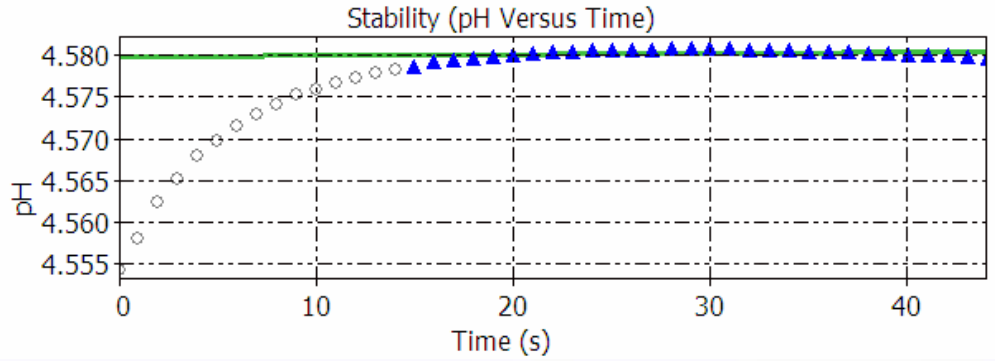
Tolmetin

Starting from solid





LogS = -4.17



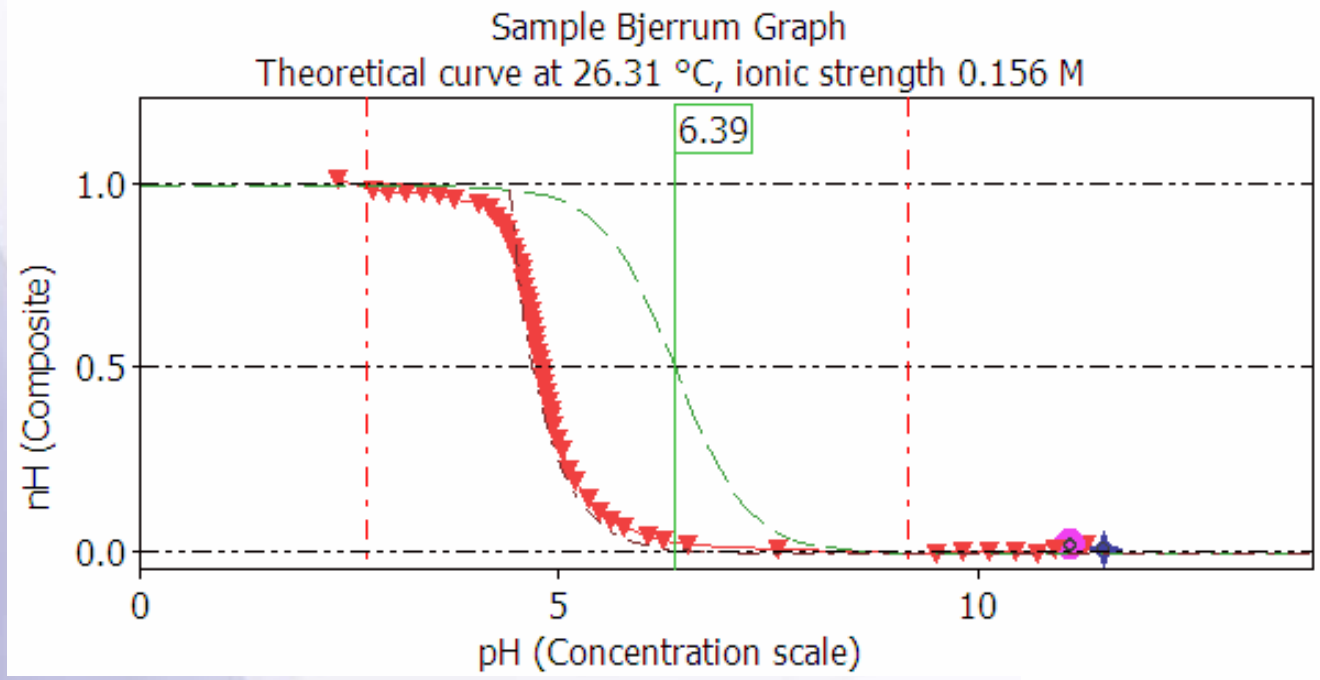
Experiment time: 90mins

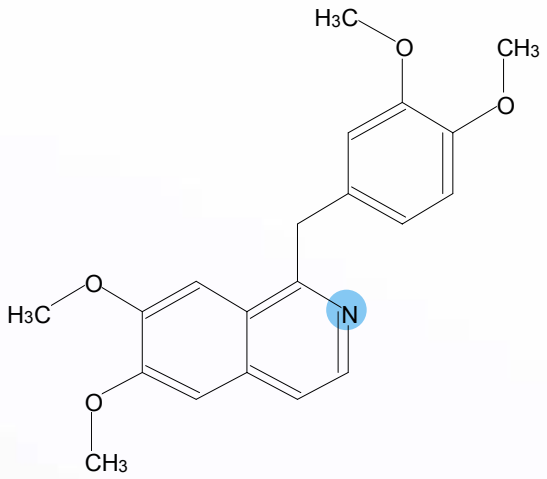
Average wait: ~50s

“Classic Method” – Super Chaser

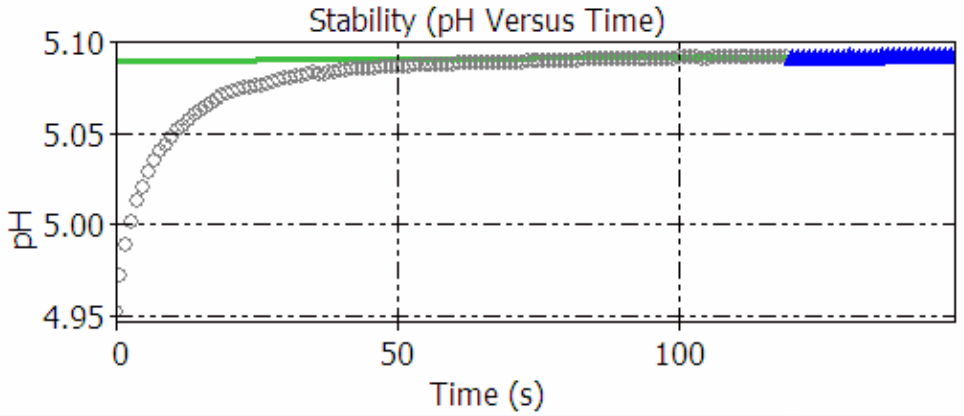
Papaverine

Starting from solid





LogS = -4.43



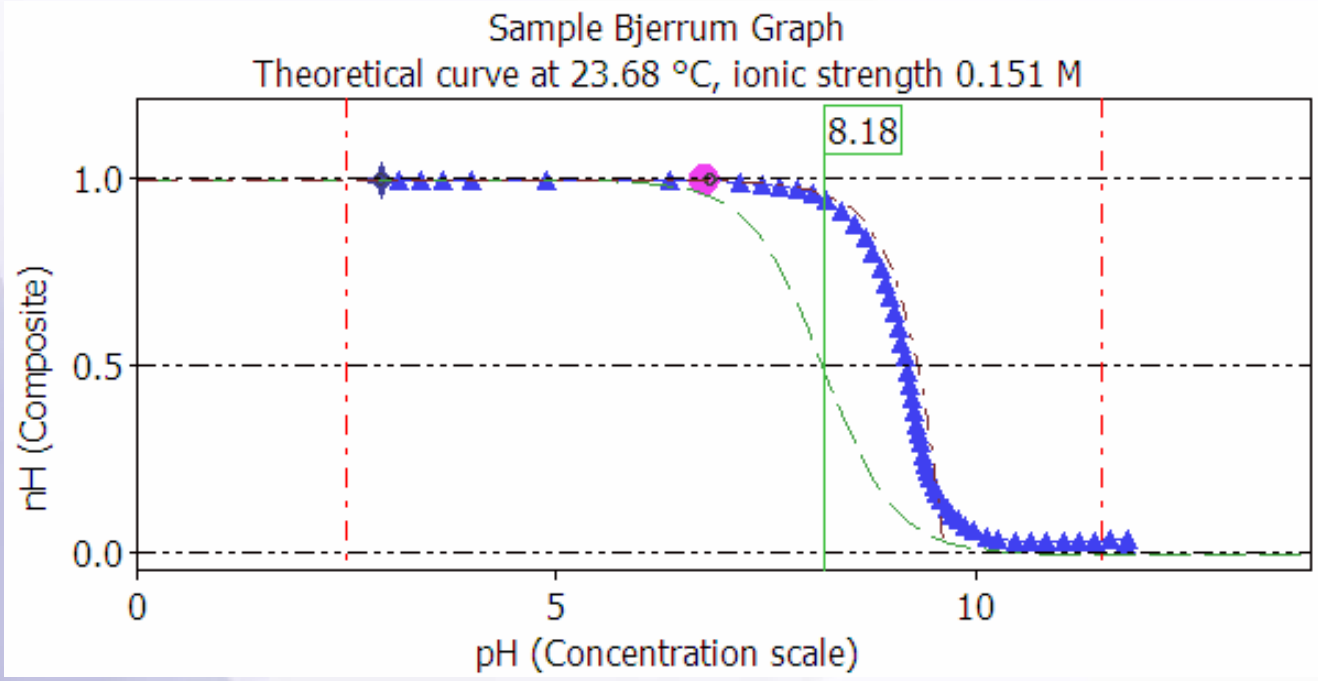
Experiment time: 100mins

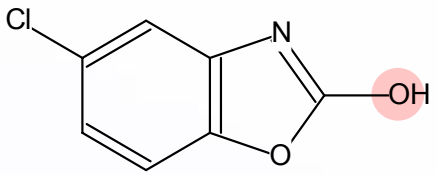
Average wait: ~150s

“Classic Method” – Super Chaser

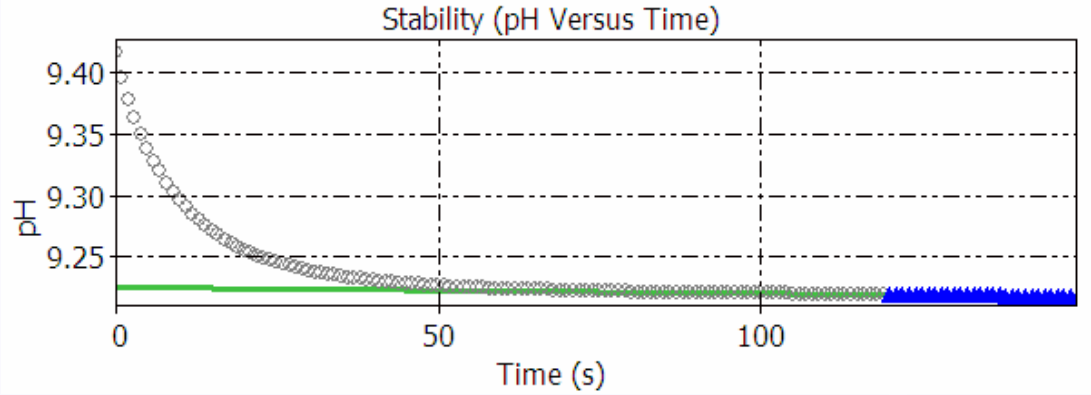
Chlorzoxazone

Starting from solid





LogS = -2.97



Experiment time: 122mins

Average wait: ~150s

Conclusions and Future Research

Real time monitoring of precipitation/dissolution effects during the CheqSol assay yields interesting insights into the solution chemistry of ionisable drugs.

Future Research:

- Can we find any “ghost” molecules?
- Can we relate the precipitation/dissolution behaviour to structural properties and/or bioavailability?
- Compare our data against traditional dissolution measurements.
- Are they any other techniques that measure precipitation rates?
- What impact does this information have on formulation design?

CheqSol Papers

- Stuart, M. Box, K. **Chasing equilibrium: measuring the intrinsic solubility of weak acids and bases**. Anal. Chem. **2005**, 77(4), 983-990
- Box, K J. Völgyi, G. Baka, E. Stuart, M. Takács-Novák, K. Comer, J E A. **Equilibrium vs. kinetic measurements of aqueous solubility, and the ability of compounds to supersaturate in solution - a validation study**. J. Pharm. Sci. **2006**, 95, 1298-1307.
- Sköld, C. Winiwarter, S. Johan Wernevik, J. Bergström, F. Engström, L. Allen, R. Box, K. Comer, J. Mole, J. Hallberg, A. Lennernäs, H. Lundstedt, T. Ungell, A-L. Karlén, A. **Presentation of a Structurally Diverse and Commercially Available Drug Data Set for Correlation and Benchmarking Studies**. J. Med. Chem. 2006, 49(23), 6660-6671
- Llinàs, A. Burley, J C. Box, K J. Glen, R C. Goodman, J M. J. **Diclofenac Solubility: Independent Determination of the Intrinsic Solubility of Three Crystal Forms**. Med. Chem.; 2007, 50 (5), 979-983 *Collaborative research with the University of Cambridge*
- Llinàs, A. Box, K J. Burley, J C.Glen, R C. Goodman, J M.J. **A new method for the reproducible generation of polymorphs: two forms of Sulindac with very different solubilities.** J. Applied Crystallography, 2007, 40(2), 379-381. *Collaboration with the University of Cambridge.*