



Solubility-pH profile of drugs.
**Experiences and surprises in logS
measurements**

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Overview of presentation

- **new protocol of saturation shake-flask method**
- **revisit of Henderson-Hasselbalch (HH) relationship**
- **surprising experiences in solubility determination**

Development of physico-chemical profiling

1971 lipophilicity, ionization

Leo, Hansch, Elkins: Chem. Rev. 71, 525-616 (1971)

1995 solubility, permeability (introduction of BCS)

Amidon, Lennernas, Shah, Crison: Pharm. Res. 12, 413-420 (1995)

2002 pharmaceutical profiling

Kerns, Di: Curr. Top. Med. Chem. 2, 87-98 (2002)

- solubility
 - ionization
 - lipophilicity
 - permeability
- +
- integrity
 - stability
 - metabolism
 - protein binding
 - CYP-450 inhibition

The importance of solubility in drug research

- important molecular property that influences the intestinal absorption → determines bioavailability
- useful during lead selection and optimization and serves as a screening parameter
- required for biopharmaceutical classification (BCS)
- necessary for salt selection and optimization of formulation

Boom in solubility research from 90-ies

- **new solubility determination methods**
 - **demand for standardization**
 - **claim for HT approaches**
 - **claim for compound-sparing assays**
 - **affords for *in silico* prediction**
- but the largest need is a better understanding of solubility reactions, particularly in case of ionizable molecules in order to give correct interpretation of experimental data

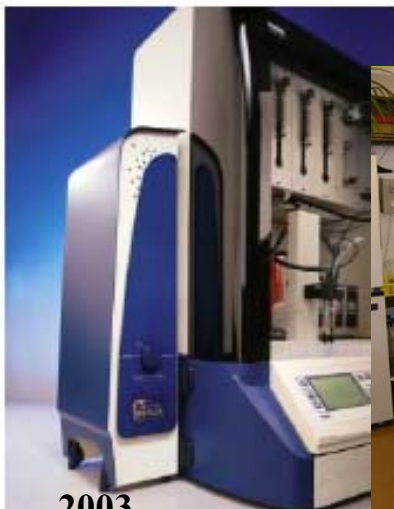
Physico-chemical profiling at Semmelweis Univ.

➤ We started with logP measurement (1977)

- **basic research: lipophilicity of amphoteric drugs
ion-pair partition**
- **method development in collaboration with Sirius
(1992-)**
- **we measured logP values for pharm. companies**



1994



2003



logP, pK_a, S, P_e

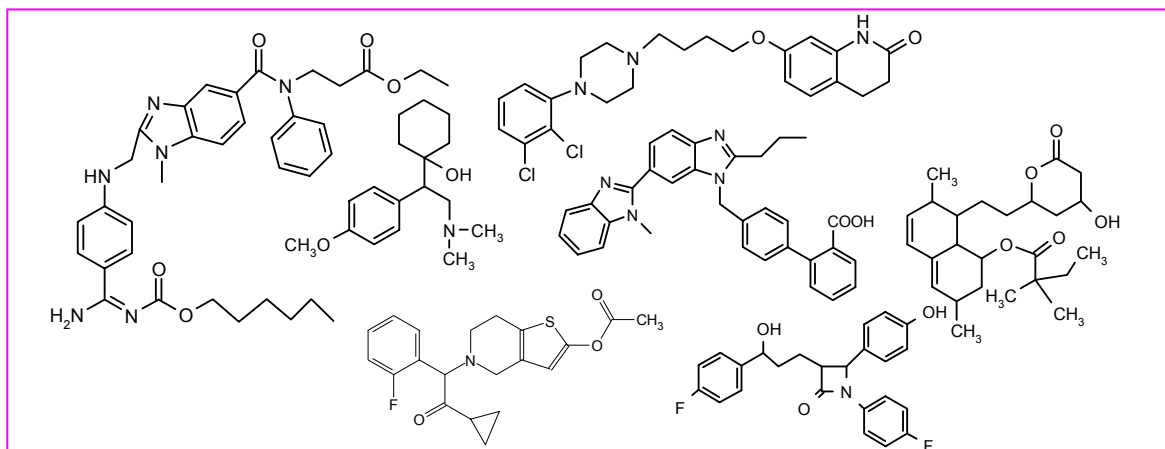
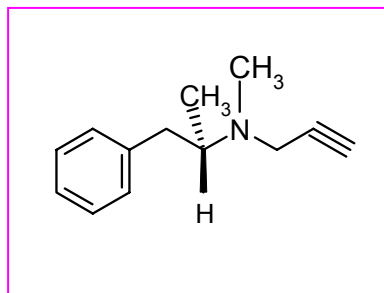
How we involved in solubility measurement?

- We got the first request for solubility determination in 1990

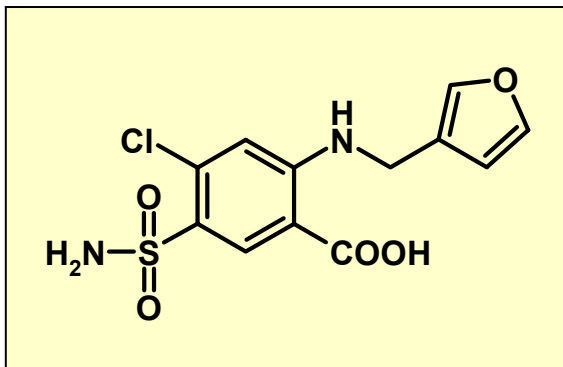
sample: deprenyl (Jumex[®]) (Chinoïn)

method: saturation shake-flask (SF)

- In the last decade we measured more logS than logP
more samples → more experiences → more surprises

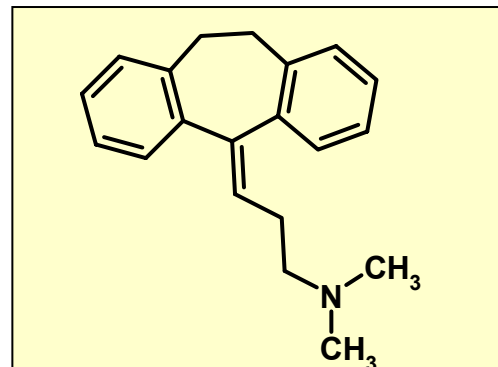


Why it is difficult to estimate the solubility?



$\log P$: 2.56

$\log S$: -4.39



$\log P$: 5.04

$\log S$: -4.39

➤ Solubility is affected by:

- particle size
- crystallinity
- polymorphism
- aggregation, micelle formation
- adsorption on the solid excess, etc.

We use these solubility definitions:

➤ **Equilibrium (= thermodynamic) solubility (S)**

the concentration of compound in a saturated solution when solid is present and solution and solid are at equilibrium

➤ **Intrinsic solubility (S_0)**

the equilibrium solubility of the free acid or base form of an ionizable compound at a pH where it is fully unionized

➤ **Apparent solubility (S_{pH})**

the equilibrium solubility of an ionizable compound at a pH where ionized form is also present

➤ **Solubility of salt (S_{salt})**

$$S_{\text{salt}} = \sqrt{K_{\text{sp}}}$$

We use these methods:

➤ **Saturation shake-flask (SF)**

temperature: controlled (25.0 or 37.0 °C)

medium: Britton-Robinson or Sørensen buffer

phase-separation: sedimentation

conc. measurement: UV spectroscopy

➤ **Chasing Equilibrium Solubility Method (CheqSol)**

potentiometric approach

GLpKa (Sirius Anal. Instr. UK)

Stuart, Box: Anal Chem. 77, 983-990 (2003)

Box, Völgyi, Baka, Stuart, Takács-Novák, Comer: J. Pharm. Sci. 95. 1298-1307 (2006).



New protocol of SF method

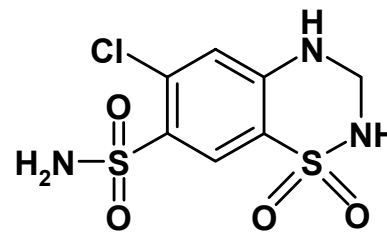
Aim of the work:

- to investigate the critical experimental conditions of SF method
- to reveal their influence on intrinsic solubility
- to create a standardized protocol

Factors studied:

- composition of buffer
- amount of solid excess
- temperature
- equilibration time
- technique of phase-separation

Model compound:



- ionizable
- sparingly soluble
- stable
- UV active

Results

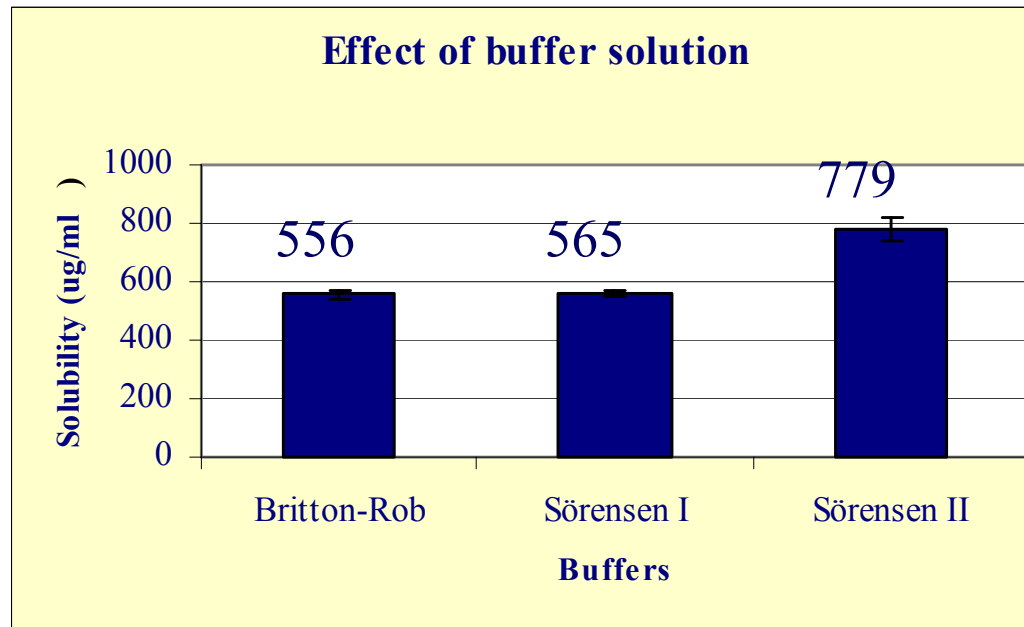
S_0 : $556 \pm 13.2 \mu\text{g/ml}$ $n=18$

pH: 6.0

pK_{a1} : 8.75 pK_{a2} : 9.88

➤ effect of buffer solution

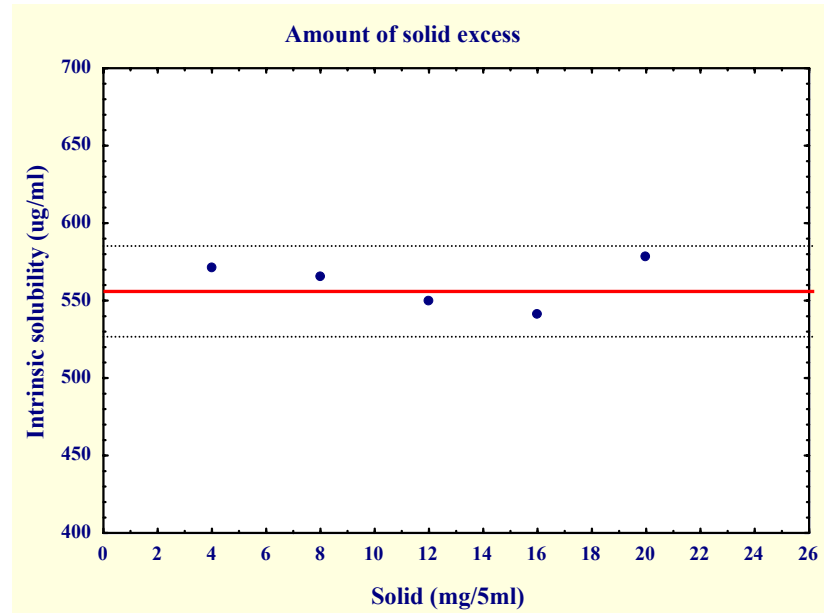
1. Britton-Robinson
($I=0.089$)
2. Sörensen-phosphate (I)
($I=0.076$)
3. Sörensen-citrate (II)
($I=0.318$)



➤ the S_0 is influenced by the ionic strength of the buffer, it is related to the changes in the activity coefficient (Streng et al. J. Pharm. Sci. 1984)

➤ effect of solid excess

the amount of solid weighted was from 4 mg to 20 mg by 4 mg steps



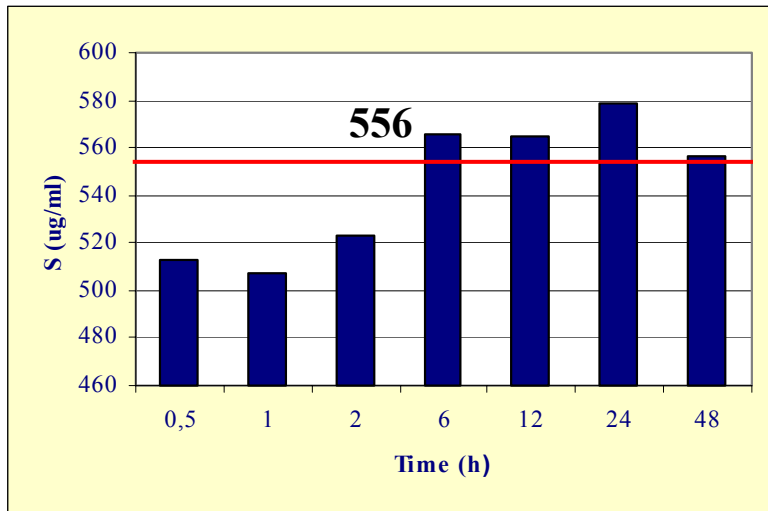
➤ the S_0 is not influenced by the solid excess

we recommend using small but sufficient excess ($\sim 5-10$ mg/5ml) to avoid the difficulties in sampling

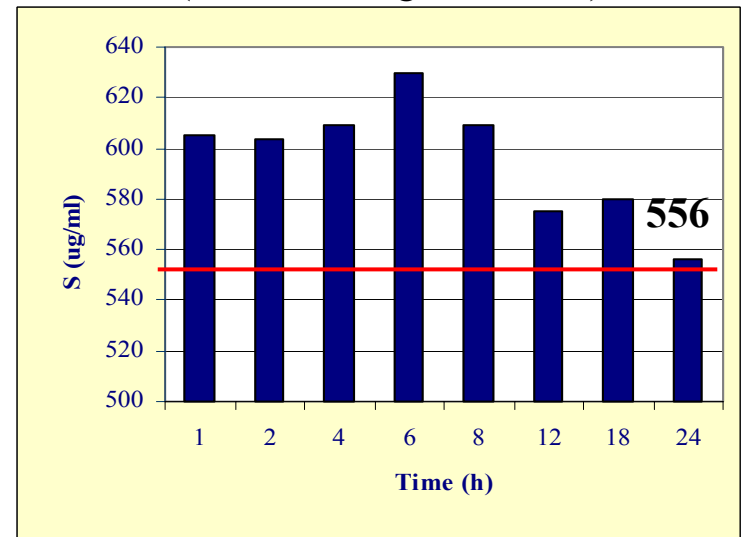
➤ effect of equilibration time

- stirring (agitation)
- sedimentation (phase-separation)

Impact of stirring-time
(when sedimentation: 24 hours)



Impact of sedimentation-time
(when stirring: 48 hours)



➤ equilibration time: $6 + 18 = 24$ hours

New protocol of SF method:

- **buffer:** B-R (pH 2.5 – 11.5) or Sörensen-phosphate (pH 3-7)
- **temperature:** 25°C or 37°C (± 0.1)
- **solid excess:** small excess (~ 5 -10 mg/5ml)
- **incubation time:** 24 hours (6 + 18)
- **phase-separation:** sedimentation
- **concentration measurement:** UV spectroscopy

- it allows to determine equilibrium solubility faster, less than 36 hours
- it was applied in the last 3 years at more than 50 compounds and only one necessitated longer equilibration time



Revisit of HH relationship

- **the pH-dependence of equilibrium solubility of ionizable molecules is described by HH equation**

$$\text{HA} \quad \log S_{pH} = \log S_0 + \log(1 + 10^{pH - pK_a})$$

$$\text{B} \quad \log S_{pH} = \log S_0 + \log(1 + 10^{pK_a - pH})$$

- **the HH relationship can be used to predict the solubility at physiologically relevant pHs (1.5, 5.5, 6.8, 7.4) if pK_a and $\log S_0$ are known**
- **validity of HH equation has been widely investigated**



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Accuracy of calculated pH-dependent aqueous drug solubility[☆]

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Abstract

The aim of the present study was to investigate the extent of the pH-dependent aqueous solubility of cationic drugs. The solubility was determined with a small-scale shake flask method. Each set of solubility curves were obtained using at least 10 different pH values reflecting the pH of the bulk and acid microclimate for all compounds. The experimental study revealed a large pH-dependent solubility interval, which is in sharp contrast to the range of solubility between the completely uncharged and ionized forms. The range of solubility between the completely uncharged and ionized forms was only 1.1 log units, whereas that for amiodarone was 3.5 log units. In conclusion, the investigated cationic drugs display a large pH-dependent solubility interval. The Henderson-Hasselbalch equation in many cases will only give rough estimations of the solubility. © 2004 Elsevier B.V. All rights reserved.

Keywords: Solubility; Henderson-Hasselbalch; Cationic drugs; pH-dependent solubility

- investigated 25 amines
- commented limited applicability



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Solubility of sparingly-soluble ionizable drugs[☆]

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Abstract

The experimental and computational basis of the pH-dependent measurement of solubility of sparingly-soluble ionizable drugs is reviewed. Recently described compound-sparing (but still accurate) approaches, suitable for application in preclinical development, and appropriate for the analysis of solubility of “problematic” molecules, are critically examined. A number of useful experimental methods are reviewed, including the miniaturized shake-flask microtitre plate, the micro solubility self-calibrating direct UV, potentiometric, and the micro dissolution methods. Several molecules were selected as case studies to illustrate important concepts, with re-analysis of literature data using recently established computational tools.

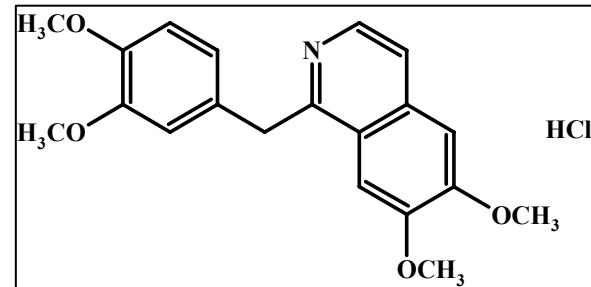
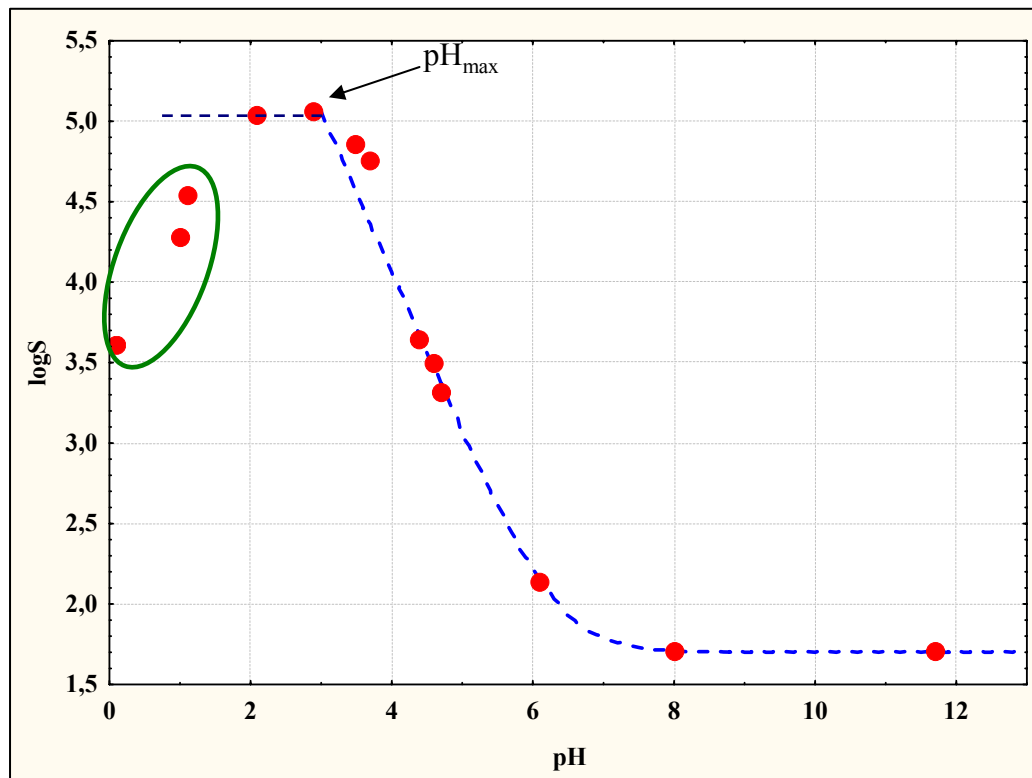
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Keywords: Sparingly-soluble ionizable compounds; pH-dependent solubility; Solubility equations; Aggregation; Complexation; Shake-flask method; Miniaturized shake-flask method; Dissolution template titration method; Micro dissolution method

- computational basis
- re-analyzed literature data

- **we performed a systematic study using structurally diverse compounds**
 - 4 monoprotic bases**
 - 1 diprotic base**
 - 1 ampholyte**
- **we measured**
 - **pK_a values**
 - **logS₀ values by two methods (SF, CheqSol)**
- **we determined the logS_{pH} values over a wide pH range (0-12) by SF method**

Papaverine hydrochloride



Exp. conditions:

method: SF new protocol

temp.: 25.0 °C

Results:

pK_a : 6.36

S_o : 17 $\mu\text{g/ml}$ (SF)

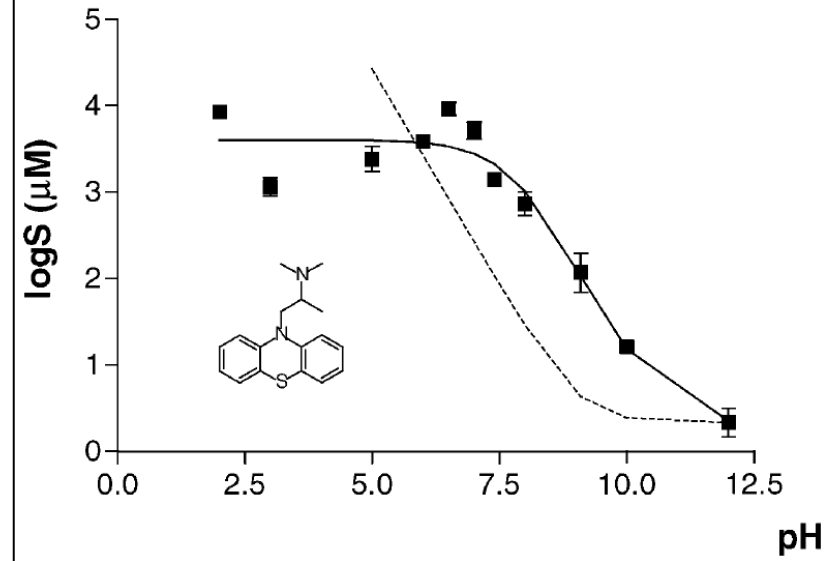
17 $\mu\text{g/ml}$ (CheqSol)

$\log S_o$ [μM]: 1.70

➤ HH valid

➤ $\text{pH} < 2$ common-ion effect

Promethazine



Bergström (2004)

Exp. conditions:

method: small-scale SF
medium: phosphate buffer
temp.: $22.5 \pm 1^\circ\text{C}$

Results:

pK_a : 9.1
 $\log S_o$: 0.3

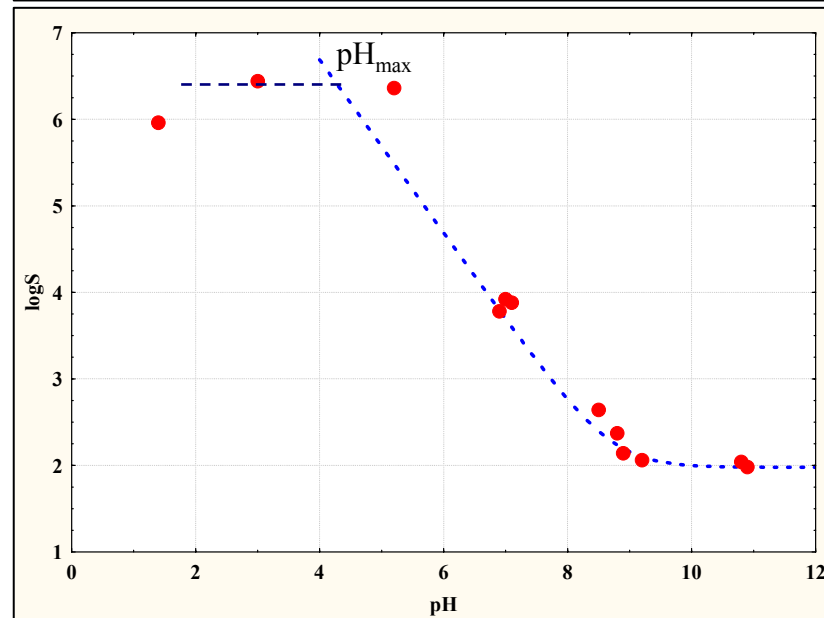
Our

Exp. conditions:

method: SF new protocol
medium: BR buffer
temp.: $25.0 \pm 0.1^\circ\text{C}$

Results:

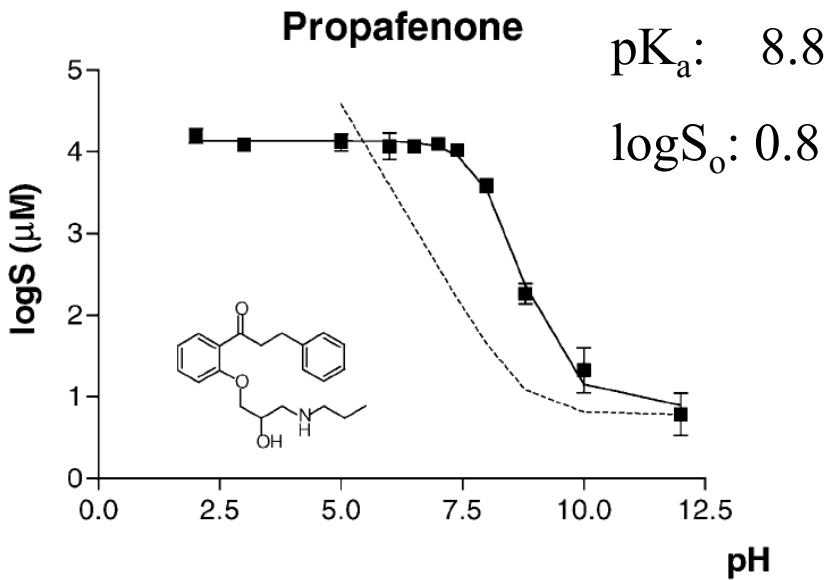
pK_a : 8.71
 $\log S_o$: 1.98



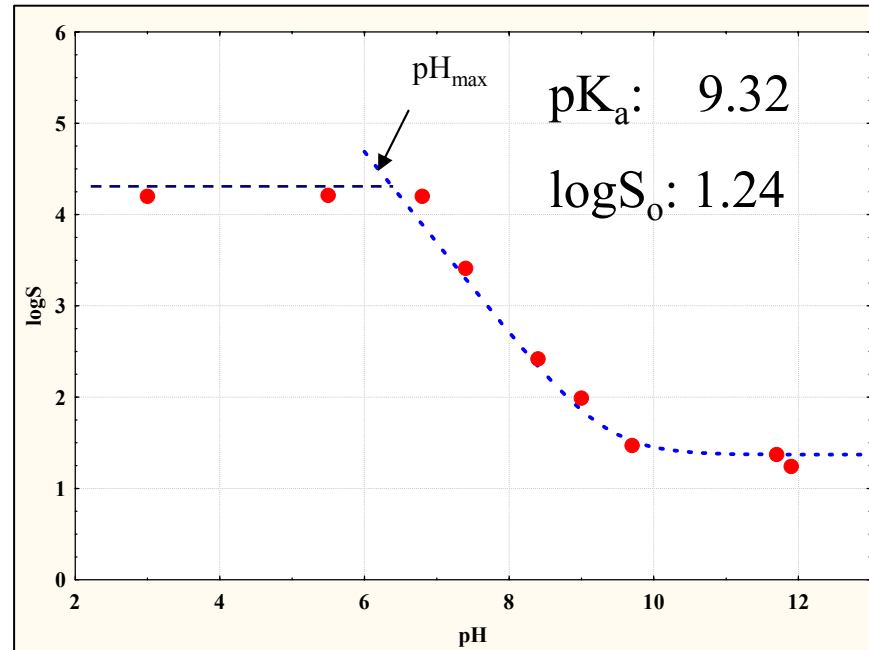
➤ HH valid

➤ $\text{pH} < 2$ common-ion effect

Bergström (2004)

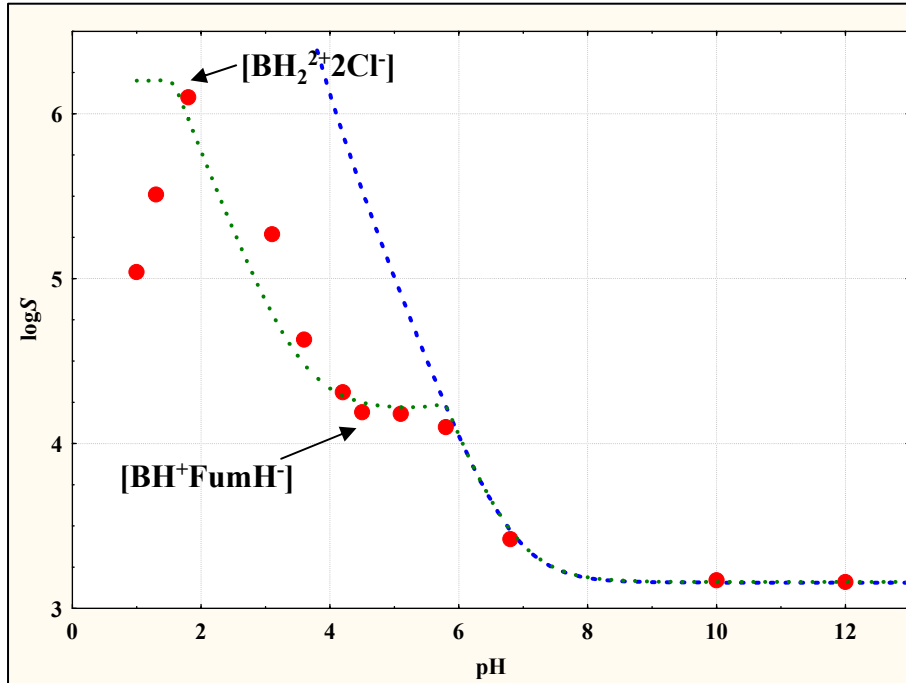
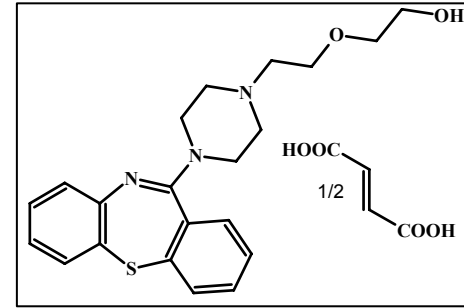


Our

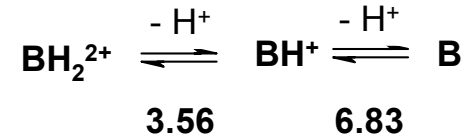


- HH relationship is valid if accurate pK_a and $\log S_0$ values are used
- aggregation may alter the curve but *before* proposing aggregation/micelle formation etc., the experimental data should be scrutinized and tested rigorously

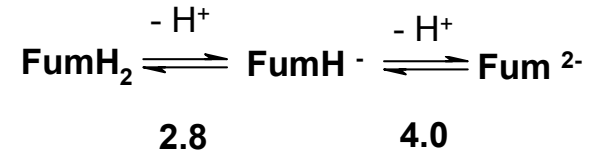
Quetiapine hydrogen fumarate



diprotic base:



fumaric acid:



blue line: theoretical HH curve

green line: model includes salt solubility

- HH valid up to pH 6
- at pH 2 dichloride salt is ppt
- pH < 2 common-ion effect

What we have learnt from this study?

- the experiments must be carefully designed and performed
- the most critical point is the precise pH control during the measurement (before and after the incubation), otherwise false conclusions can be drawn
- the common-ion effect below pH 2 is significant even in case of non-hydrochloride salts
- deviation from HH relationship often due to the inaccuracy of the applied method
- before supposing special molecular interactions and applying complicated equations for calculation the experiments must be checked



Surprising experiences

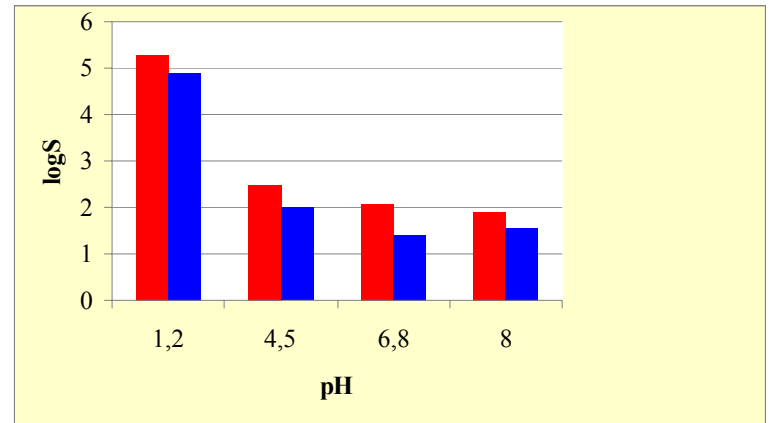
Example 1.

2 samples: free base $pK_a: 5.66$

hydrochloride salt

S_{pH} at pHs: 1.2, 4.5, 6.8 at 37 °C

pH	S_{pH} [$\mu\text{g/ml}$]			
	1.2	4.5	6.8	8.0
base	28 650	36	10	13
HCl salt	63 300	110	45	30



Different solubility data!

➤ different crystal structures

pH: 1.2 (0.1M HCl)

pH: 4.5 – 8.0 (Sörensen)

Example 2.

2 polymorphs: A form

$pK_a: 9.63$

B form

at pHs: 1.2 - 6.8 $S_{pH} > 50\%$

pH	S_{pH} [$\mu\text{g/ml}$]		
	8.9	9.2	12
A form	1 800	1280	460
B form	2 080	1290	460



No significant difference in solubility data!

➤ A and B forms convert to the base with identical crystal structure

Example 3.

2 samples: hydrochloride salt
mesylate salt

S_{pH} at pHs: 1.2, 3.1, 4.5, 6.8 at 37 °C

difficulties:

at pH 1.2 stable supersaturated solution formed

at pH 6.8 opalescent colloid is appeared

pH	S_{pH} [$\mu\text{g/ml}$]			
	1.2	3.1	4.5	6.8
HCl salt	48 000 ± 1000	370 ± 10	13 ± 1	~ 0.5
CH ₃ SO ₃ H salt	47 000 ± 1000	360 ± 10	62 ± 5	~ 0.5



**Identical solubility
of the salts!**

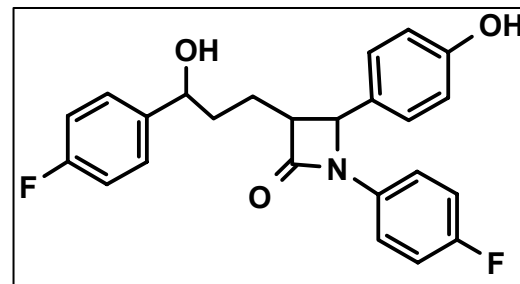
- complicated and unrevealed acid-base chemistry
- analysis of solid is necessary

Example 4.

requested solubility data:

at pHs: 1.2, 4.5, 6.8

at 37 °C



$pK_a: 9.7$

monovalent weak acid

below pH 7: unionized

$$S_{pH} = S_o$$

S_{pH} [$\mu\text{g/ml}$]			
pH: 1.2	pH: 4.5	pH: 6.8	dest. water
1.2	0.8	1.0	2.1



Management insisted on the measurement!

- understanding of ionization equilibria is useful before the solubility is ordered to measure

Summarizing

- solubility measurement requires careful experimental design and perfect understanding of solubility reactions
- care must be taken to avoid the interactions with buffer components
- the pH must be controlled before and after the incubation period
- attention has to be paid to reach the equilibrium state
- analysis of the solid at the end of the incubation can reveal molecular transformation (like polymorph transition, new salt formation)
- the interpretation of results sometimes more challenging than we realize

„Solubility still remains deceptively easy to untrained eye and quite difficult to those interested in precise data and clear interpretation of results.”

Alex Avdeef (2007)



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