



# In support of Henderson Hasselbalch – solubility-pH profiles from shake-flask correlate with profiles calculated from pK<sub>a</sub> and intrinsic solubility

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## Abstract

**Purpose:** The goal of this work was to show that the classic Henderson Hasselbalch (HH) equation is valid, and that reported discrepancies can be explained without invoking deficiencies in HH.  
**Methods:** The pK<sub>a</sub> of six ionizable drugs was measured by pH-metric and pH-UV methods. Their intrinsic solubility was also measured by the CheqSol method. These values were used in HH equations to create solubility-pH profiles. These profiles were compared with recently published values for solubility measured in buffer solutions at varying pH [1].  
**Results:** The pK<sub>a</sub> and intrinsic solubility data are shown in the six graphs. The pH-solubility profiles drawn using the HH equations derived from the new experimental data were close to the solubility values measured by shake-flask in discrete buffers, as demonstrated in these examples. Discrepancies in pH regions where the drugs were unionized may have been explained by the difficulties in the shake-flask method of measuring low concentrations at extreme pH.  
**Conclusion:** It has been shown that solubility-pH profiles drawn using the Henderson Hasselbalch equation provide an accurate picture of changes in drug solubility as a function of pH.

## The Henderson Hasselbalch equation

The Henderson Hasselbalch equation describes the derivation of pH as a measure of acidity, using pK<sub>a</sub>, the acid dissociation constant. The equation is useful for estimating the pH of a buffer solution and finding the equilibrium pH in acid-base reactions. It is most commonly written for a weak acid HA (or a weak base B) with one pK<sub>a</sub>, as

$$pK_a = pH + \log \frac{[HA]}{[A^-]} \quad pK_a = pH + \log \frac{[BH^+]}{[B]}$$

Hasselbalch published the equations above in 1917 [2] as logarithmic forms of equations published by Henderson in 1908 [3]. As the years have passed by, the term "Henderson Hasselbalch equation" has come to be used for several acid-base properties that can be described using the definitions, assumptions and rules that govern the behaviour of weak acids and bases in aqueous solutions. For example, solubility.

## Henderson Hasselbalch and solubility

Consider the solubility of a monoprotic weak base B. The solubility S is equal to the total amount of B in solution, i.e.

$$S = [BH^+] + [B] \quad 1$$

The protonation of B in solution can be written as

$$B + H^+ = BH^+ \quad 2$$

for which the ionization constant K<sub>a</sub> is written as

$$K_a = \frac{[B][H^+]}{[BH^+]} \quad \text{which rearranges to} \quad BH^+ = \frac{[B][H^+]}{K_a} \quad 3$$

Substituting for BH<sup>+</sup> in equation 1 leads to

$$S = \frac{[B][H^+]}{K_a} + [B] \quad \text{which leads to} \quad \frac{[B][H^+] + [B]K_a}{K_a} \quad 4$$

Expressing equation 4 in logarithmic form leads to the expression

$$\log S = \log \left( \frac{[B][H^+] + [B]K_a}{K_a} \right) \quad \text{which rearranges to}$$

$$\log \left( \frac{[B]}{K_a} ([H^+] + K_a) \right) \quad \text{therefore}$$

$$\log S = \log [B] + \log ([H^+] + K_a) - \log K_a \quad 5$$

The intrinsic solubility S<sub>0</sub> of a weak base may be defined as the equilibrium solubility of the free base form of an ionizable compound at a pH where it is fully un-ionized, where equilibrium solubility is the concentration of compound in a saturated solution when excess solid is present, and solution and solid are at equilibrium. From this definition, it is clear that

$$\log S_0 = \log [B] \quad 6$$

The ionization constant K<sub>a</sub> is usually expressed as a pK<sub>a</sub> value, where

$$pK_a = -\log K_a \quad 7$$

Equation 5 can therefore be rewritten as

$$\log S = \log S_0 + \log ([H^+] + K_a) + pK_a \quad 8$$

The term [H<sup>+</sup>] may be derived from the pH of the solution, since

$$pH = -\log [H^+] \quad 9$$

Thus equation 8 can be used to draw a solubility-pH profile of a monoprotic weak base using only the sample's pK<sub>a</sub> and S<sub>0</sub> (intrinsic solubility) values. Is it a Henderson Hasselbalch equation? No. But it accords with the general principles of Henderson Hasselbalch, and it is the equation used to draw the solubility-pH profiles described in this poster.

## Experimental

The pK<sub>a</sub> values of six compounds were accurately measured pH-metrically using the Sirius GLpKa instrument, and their intrinsic solubilities (logS<sub>0</sub>, in μM) were measured in aqueous solutions by Sirius CheqSol. The pK<sub>s</sub> for two compounds were confirmed by pH-UV techniques measured using the Sirius D-PAS. All pK<sub>s</sub> were taken from Yasuda-Shedlovsky extrapolations, using an average of 7 points per extrapolation.

## Drawing the solubility-pH profiles

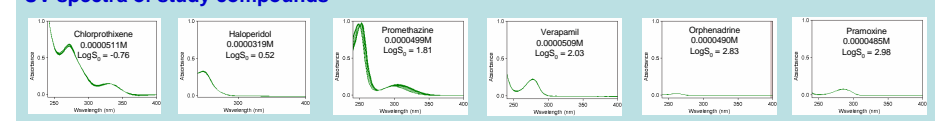
Solubility (logS) values as a function of pH were calculated using equation 8. Solubility-pH profiles were drawn, and overlaid in red over copies of published profiles [1], taking care to ensure that the scales were accurately matched. In this way the new profiles could be compared with the published profiles.

## Published data used for comparison

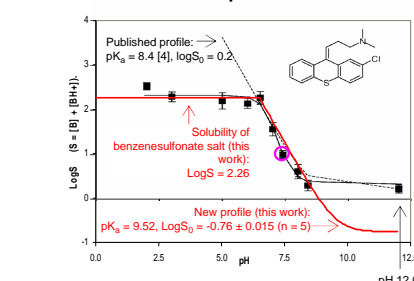
The published data [1] showed solubility measured by a shake-flask technique in phosphate buffer at 9 to 12 pH values plotted against pH, with a solid black line fitted to the data points. Profiles were calculated using an HH relationship (dotted black lines), based on published pK<sub>a</sub> values together with measured S<sub>0</sub>, which was taken to be the solubility at the highest pH where the compounds were unionized. Some of the published pK<sub>a</sub> values did not agree with the values measured in this study. This would lead to differences in the HH profiles.

S<sub>0</sub> was determined from concentrations in supernatants at high pH, measured by UV. The graphs below show UV spectra for the six compounds. Note that haloperidol, orphenadrine and pramoxine absorb UV weakly, which could lead to difficulties in concentration measurement, and hence to uncertainties in S<sub>0</sub>.

## UV spectra of study compounds

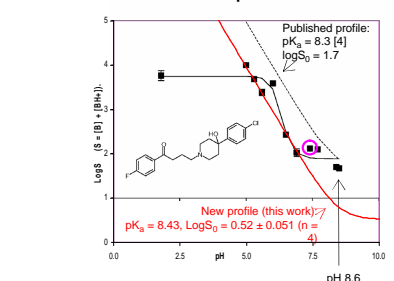


## Chlorprothixene

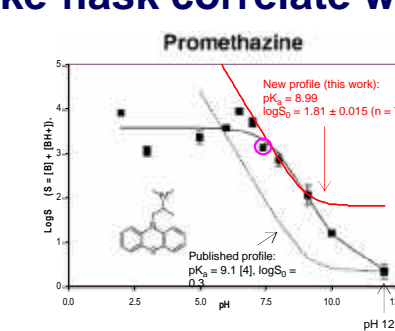


The published HH profile was calculated using a published pK<sub>a</sub> value lower than the newly-measured value, and an S<sub>0</sub> value measured at pH 12.0. The new profile fits all the mid-pH shake-flask points. The shake flask points at lower pH show a constant logS of about 2.3, presumably because the compound formed an insoluble salt. High levels of phosphate were present for the published data. In the current work the solubility of a benzenesulfonate salt was measured, and shown to fit a similar profile to that for the phosphate salt.

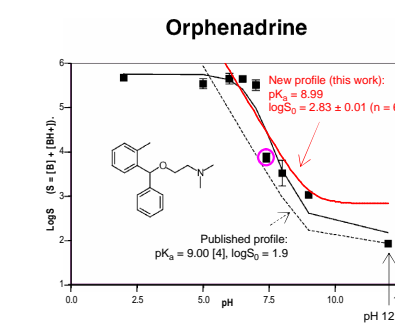
## Haloperidol



The new profile fits five of the shake-flask points very well. The S<sub>0</sub> value measured at pH 8.6 and used for the published HH profile may be too high. Additional shake-flask data at higher pH would have been useful. Haloperidol is difficult to get into aqueous solution at low pH because its salts are poorly soluble. In this work, haloperidol was dissolved in aqueous solution at low pH by sonicating at about 60°C for up to 10 minutes.



The new profile fits the shake-flask data quite well at mid-range pH. Shake-flask points on a plateau below pH7 probably represent the solubility of a phosphate salt. The published HH profile was based on an S<sub>0</sub> value which may be too low, measured at pH 12.1.



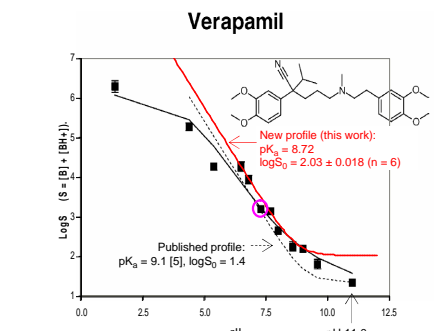
The new profile fits the shake-flask data quite well at mid-pH. Additional shake-flask data at higher pH would be useful. Orphenadrine has low UV absorbance.

## Discussion

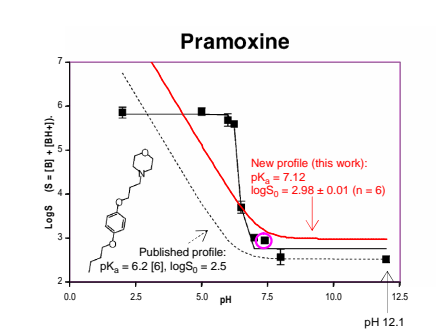
Solubility-pH profiles of six bases drawn using HH principles from new measured values for pK<sub>a</sub> and S<sub>0</sub> appear to correlate well with measured shake-flask solubility in regions just below the pK<sub>a</sub>, where the compounds were ionized. At lower pH the solubility appears to be controlled by the salt. Shake-flask is the reference method for solubility measurement. However, it appears risky to draw solubility-pH profiles of bases using S<sub>0</sub> measured at high pH, if samples have low UV absorbance.

## References

- [1] Bergström, C A S, Luthman, K, Artursson, P. Accuracy of calculated pH-dependent aqueous drug solubility. Eur. J. Pharm. Sci., 2004, 22, 387-398.
- [2] Hasselbalch, K A. Die Berechnung der Wasserstoffzahl des Blutes aus der freien und gebunden Kohlensäure desselben, und die Sauerstoffbindung des Blutes als Funktion der Wasserstoffzahl. Biochemische Zeitschrift, 1917, 78, 112-144.
- [3] Henderson, L J. Concerning the relationship between the strength of acids and their capacity to preserve neutrality. Am. J. Physiol., 1908, 21, 173-179.
- [4] Encyclopedia of Therapeutic Drugs, Churchill Livingstone, New York, 1991
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The published HH profile and the new profile agree quite well at mid-pH. Although the published profile was drawn using a slightly higher pK<sub>a</sub> value, it is also based on an S<sub>0</sub> value which is slightly lower, and the two profiles agree more or less over most of the pH range.



Neither profile fits the shake-flask data well. The UV absorbance of pramoxine is low, and concentrations would be difficult to measure. Note that the red curve is drawn using pH-metric data, and does not depend on UV spectroscopy.

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