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Original Scientific Article

pK_a-critical Interpretations of Solubility–pH Profiles: PG-300995 and NSC-639829 Case Studies

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Abstract

Two weak bases, PG-300995 (anti-HIV agent) and NSC-639829 (anti-tumor agent), whose log S – pH profiles had been previously published, but whose pK_a values had not been reported, were analyzed using a method which can determine pK_a values from log S – pH data. This "S_{pH}-pK_a" technique, although often practiced, can result in inaccurate pK_a values, for a variety of reasons. The operational S_{pH}-pK_a values were compared to those predicted by MarvinSketch (ChemAxon), ADMET Predictor (Simulation Plus), and ACD/Percepta (ACD/Labs). The agreement for the sparingly-soluble PG-300995 was reasonably good. However, a substantial difference was found for the practically-insoluble NSC-639829. To probe this further, the pK_a of NSC-639829 was measured by an independent spectrophotometric cosolvent technique. The log S - pH profile of NSC-639829 was then re-analyzed with the independently-measured pK_a. It was found that the equilibrium model which best fit the solubility data is consistent with the presence of a monocationic NSC-639829 dimeric species below pH 4. This illustrates that an independently-determined accurate pK_a is critical to mechanistic interpretations of solubility-pH data. Apparently, the Henderson-Hasselbalch equation holds for PG-300995, but not NSC-639829.

Keywords

sparingly-soluble; solubility as a function of pH; solubility equations; shake-flask solubility; aggregation

Introduction

It is possible to determine the ionization constant (pK_a) of an ionizable molecule from its solubility-pH profile (log *S* - pH), provided the curve is accurately predicted by the well-known Henderson-Hasselbalch equation [1] (cf., Appendix). This simple pK_a-from-solubility (S_{pH}-pK_a) method has been popularized by Zimmermann [2], and has been practiced often, as noted in the critical pK_a compilations by Prankerd [3]. However, the S_{pH}-pK_a method can be significantly inaccurate when sample molecules in saturated solutions react with buffer components to form water-soluble complexes, or with each other to form water-soluble aggregates/oligomers (dimers, trimers, … [4]) or micelles with large aggregation numbers [5]. Ionizable molecules that are surface-active (e.g., long-chain acylcarnitines [5], prostaglandins [6]) or that have strong acid-base hydrogen potentials (e.g., oxytetracycline [7], cefadroxyl [8]) have a tendency to form self-aggregates and/or micelles in saturated aqueous solutions

[9,10], especially under the high concentrations used to characterize drug-salt precipitates [11,12]. These complications are not taken into account in S_{pH}-pK_a determinations.

Reliable and widely-available pK_a methods have been developed over the last two decades to measure the pK_a of practically-insoluble molecules, under conditions where saturation is avoided and self-aggregation is minimized [13,14]. Potentiometric and UV methods are particularly well-suited for measuring pK_a values. The Yasuda-Shedlovsky [15] and Origin-Shifted Yasuda-Shedlovsky [9] methods employing cosolvents have been amply demonstrated to determine accurate pK_as of molecules, some with solubility as low as 2 pg/mL (amiodarone[11,12]).

In this study we considered two weak bases (Fig. 1), PG-300995 (anti-HIV agent) and NSC-639829 (anti-tumor agent), whose log *S* – pH profiles have been published by Ran *et al.* [16] and Jain *et al.* [17], respectively.

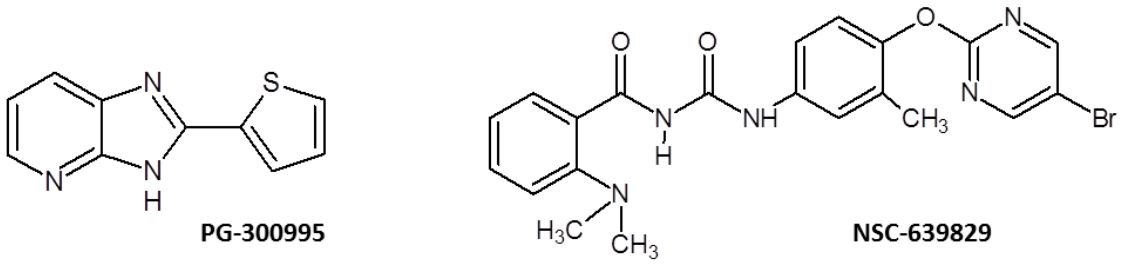

Figure 1. Structures of the molecules considered.

In these cases, the pK_a values of the molecules had not been reported. The undistorted shapes [9] of the two log *S*-pH profiles suggested that the simple weak-base Henderson-Hasselbalch equation could be used to describe the solubility-pH relationship. The data were first analyzed by the S_{pH}-pK_a method, using the computer program *p*DISOL-X (*in-ADME* Research) [10,12,18]. These operational pK_a values were compared to those predicted using MarvinSketch (ChemAxon), ADMET Predictor (Simulation Plus), and ACD/Percepta (ACD/Labs). With PG-300995 (intrinsic solubility, S_0 = 51 µg/mL [16]), the agreement was reasonably good with two of the three prediction programs. However, with the practically-insoluble NSC-639829 (S_0 ≈ 30 ng/mL [17]), there was a substantial differences between the prediction and the value determined by the S_{pH}-pK_a method. To address the apparent inconsistency, it was decided to measure the pK_a of NSC-639829 using dedicated instrumentation which can determine pK_a values to high precision. Using the measured pK$_a$, the log *S* - pH profile of NSC-639829 was rationalized with a monocationic dimeric species, predominating below pH 4. (We could not obtain samples of PG-300995 to do the experimental pK_a determination.)

Experimental

Materials

NSC-639829 (>99 % purity) was synthesized by the National Cancer Institute and was used as received in the Jain *et al.* [17] study. In the Ran *et al.* [16] study, PG-300995 was a gift from Proctor and Gamble. Methanol was purchased from Sigma-Aldrich, St. Louis, MO, USA. Solutions and solvent mixtures were made with distilled water purchased from EMD Millipore, Billerica, MA, USA. Analytical grade potassium hydrogen phthalate and KCl were purchased from Acros Organics, New Jersey, USA. The ionic strength of water was adjusted to 0.15 M with KCl. The

base titrant was prepared by diluting CO_2-free KOH concentrate (Fluka Analytical, St. Louis, MO, USA) to 0.5 M. It was standardized by titration against potassium hydrogen phthalate. The acid titrant, standardized 0.5 M HCl, was purchased from Sigma-Aldrich St. Louis, MO, USA.

Legacy Solubility Data

The Ran *et al.* [16] and Jain *et al*. [17] shake-flask solubility protocols were very similar. Briefly, excess solid was added to 1-2 mL of buffer solution. Enough NaCl had been added to the buffers to produce a total ionic strength, I = 0.2 M. The suspensions were agitated mildly for 5 d at 23 °C for NSC-639829 and 7-10 d at 25 °C for PG-300995. At the end of the stirring period, pH was read, after which the solutions were filtered and the concentrations were determined by HPLC. Mostly 10 mM buffers were used: glycine/HCl for pH < 3, citrate for pH 3-5, phosphate for pH 5-8, and glycine/NaOH for pH > 8.

The data used in this study were digitized from the log *S* – pH plots in the original publications (11 and 13 pH points for PG-300995 and NSC-639829, respectively).

Spectroscopic Measurement of the pK_a of NSC-639829

The pK_a was determined at 25 ± 0.1 °C using the SiriusT3 UV spectroscopic (UV-metric) method. The sample was added to a SiriusT3 vial as 5 microliters of 10 mM DMSO stock solution and was initially titrated in a Fast-UV pK_a screening assay. The sample was titrated from pH 2 - 12 at concentrations of 32 - 16 μM under methanol-water co-solvent conditions. Only one pK_a, with a value of 3.7, was observed. Consequently, 5 microliters of a 10 mM DMSO stock solution of NSC-639829 were dispensed into a SiriusT3 vial and the sample was titrated in a triple titration from pH 1.5 - 5.0 at concentrations of 29 - 14 μM in three different ratios of methanol-water cosolvent. The average methanol concentration was 40.9, 30.2 and 21.6 % w/w in the first, second and third titrations, respectively. The presence of cosolvent caused the pK_a to shift from its aqueous value. The apparent pK_a in the presence of cosolvent is referred to as the p$_s K_a$. Multi-wavelength UV spectra (200 - 750 nm) and pH data were collected every 0.2 pH units throughout each titration. The SiriusT3 Refine software used target factor analysis (TFA) to rationalize the 3D matrix of absorbance vs wavelength vs pH. The p$_s K_a$ values for each titration were determined as the point at which the rate of UV absorbance change was greatest across the selected wavelength range as a function of pH. The aqueous pK_a was determined by Yasuda-Shedlovsky extrapolation of the p$_s K_a$ values from each titration. There was no evidence of significant sample impurities in the pK_a determination.

Predicted pK_a

Values of pK$_a$ were predicted using MarvinSketch™ 5.3.7 (ChemAxon Ltd., Budapest, Hungary; www.chemaxon.com), and corroborated with ADMET Predictor™ 7.0 (Simulation Plus, Inc., Lancaster, CA, USA; www.simulations-plus.com), and ACD/Percepta™ 14 (ACD/Labs, Toronto, Canada; www.acdlabs.com). The predictions were used as a guide for some of the S_{pH}-pK_a analysis.

Refinement of Intrinsic Solubility, Aggregation Constants, and S_{pH}-pK_a

Detail of the mathematical approach in the *p*DISOL-X (*in-ADME* Research) computer program has been described by Völgyi *et al.* [18]. Briefly, the data analysis method uses measured log *S* - pH, along with standard deviations, SD (log *S*), as input into the *p*DISOL-X program. An algorithm was developed which considers the contribution of all species proposed to be present in solution, including all buffer

components (e.g., citrate, phosphate, glycine). The approach does not depend on any explicitly derived extensions of the Henderson-Hasselbalch equations. The computational algorithm derives its own implicit equations internally, given any practical number of equilibria and estimated constants, which are subsequently refined by weighted nonlinear least-squares regression [9,18]. Therefore, in principal, drug-salt precipitates, -aggregates, -complexes, - bile salts, -surfactant can be accommodated [4,9,18]. Presence of specific buffer-drug species can be tested. The program assumes an initial condition for the suspension of the solid drug in the buffer solution, ideally with the compound remaining saturated over a wide range of pH. First, the program calculates the volume of acid titrant that would lower the pH of the suspension to ~0. From there, a sequence of perturbations with standardized NaOH is simulated, and solubility calculated at each point (in pH steps of 0.005-0.2), until pH ~ 13 is reached. The ionic strength is rigorously calculated at each step, and pK_a values (as well as solubility products, aggregation and complexation constants) are accordingly adjusted [9]. Also, the pH electrode parameters are adjusted for the changing ionic strength [9].

At the end of the pH-speciation simulation, the calculated log *S* vs. pH curve is compared to measured log *S* vs. pH. A log S-weighted nonlinear least squares refinement commences to refine the proposed equilibrium model, using analytical expressions for the differential equations. The process is repeated until the differences between calculated and measured log S values reach a stable minimum, as described elsewhere in detail [9,18].

Results and Discussion

The aqueous pK_a value was determined by Yasuda-Shedlovsky extrapolation to be 3.76 ± 0.03. Figure 2a shows the Yasuda-Shedlovsky plot of p$_s K_a$ + log [H_2O] vs. 1000/dielectric constant. The spectra measured during titration in 40.9 % methanol are shown in Figure 2b. Each line represents a spectrum measured at a particular pH between 1.5 and 5.2.

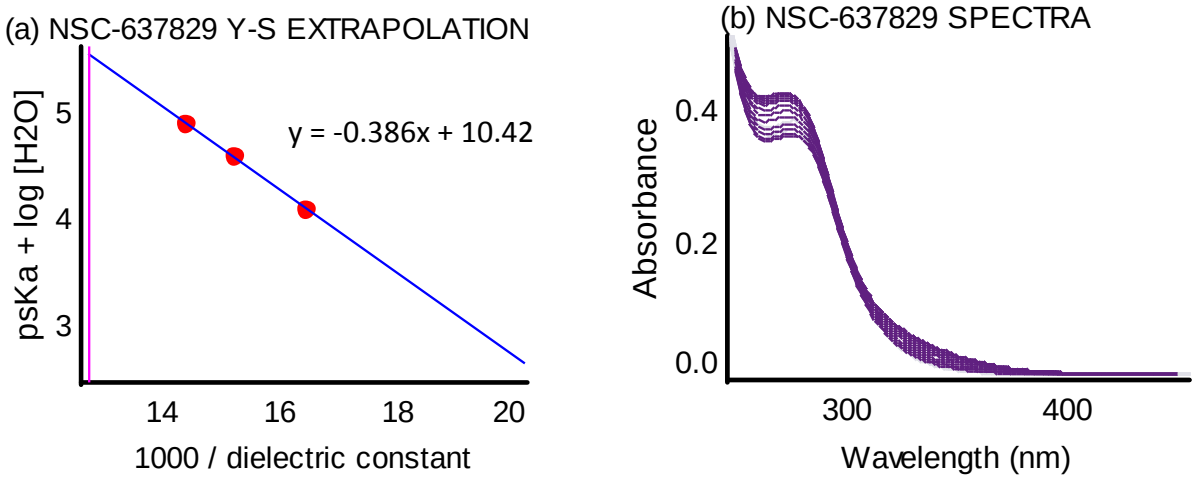

Figure 2. (a) Yasuda-Shedlovsky extrapolation of p$_s K_a$ (+ log [H_2O]) at three ratios of methanol to water. Aqueous pK_a determined from intercept with vertical red line, equivalent to 1000/dielectric constant for water, minus the log [H_2O]. **(b)** Spectra measured during titration in 40.9% methanol. Each line represents spectrum measured at a different pH between 1.5 and 5.2.

The pK_a results and those of the re-analysis the Ran et al. [16] and Jain et al. [17] solubility-pH data are summarized in Table 1.

Table 1. Summary of the Results of the Re-Analysis of the PG-300995 [16] and NSC-639829 [17] Data.

COMPOUND	Predicted pK$_a$ [a]	S_{pH}-pK$_a$ [b] ±SD	pK$_a$ (25°C, I =0.15 M) [c]	-log S_0 [d] ±SD (M)	S_0 ±SD (µg/mL)	mixed-charge dimer	log K_2 ±SD (M^{-1})	GOF [e]
PG-300995	3.52, <u>10.58</u>	3.61 ±0.06	n.d. [f]	3.59 ±0.03	51.3 ±0.7	--	--	0.77
NSC-639829	2.28, <u>7.48</u> [g]	4.70 ±0.12	3.76 ±0.03 [g]	7.22 ±0.06	0.028 ±0.001	$BH^+ + B = B_2H^+$	7.80 ± 0.14	1.9

[a]pK_a values predicted by MarvinSketch (ChemAxon). Underlined values refers to acidic group ionization; otherwise, the value is that of basic group ionization. [b]Refined pK_a by the S_{pH}-pK_a method. [c]Measured pK_a by UV cosolvent extrapolation from 21.6-40.9 % w/w CH_3OH-H_2O (this work). [d]S_0 = intrinsic solubility (this work). [e]GOF = goodness-of-fit [9,18]. [f]Not determined. [g]Acidic pK_a not found experimentally below pH 12 in Fig.3b. Also, preliminary UV pK_a assay from pH 2 – 12 indicated no additional pK_as within the range.

PG-300995 pK$_a$ and S$_0$

The two MarvinSketch-predicted pK_as for PG-300995 are 3.52 (benzimidazole –N= basic functionality) and 10.58 (–NH- acidic group). The corresponding amine pK_a from ADMET Predictor (average over 2 tautomers) was 3.60, in close agreement with that of MarvinSketch. However, the value from ACD/Percepta, 5.30, was less concordant. The refined S_{pH}-pK_a is 3.61 ± 0.06, indicated by the pH in the bend in the log *S* - pH curve in Figure 3a, agrees very well with the predictions from two of the commercial programs. There is no indication of the acidic ionization in the solubility profile, suggesting that the second pK_a must be greater than 9, which is consistent with the predicted value from all three pK_a prediction programs. Since the molecule is relatively simple and not too insoluble, and since the predicted basic pK_a agrees with the measured value, the refined S_{pH}-pK_a tentatively was taken to be a measure of the true pK_a. Thus, the Henderson-Hasselbalch equation is thought to be a valid description of the solubility-pH curve. This is, of course, a tentative assignment, since the independently measured value of the pK_a of PG-300995 is not available.

The refined intrinsic solubility, S_0 = 51.3 ± 0.7 µg/mL, agrees well with the reported value [16]. Each point in Figure 3a was assigned the standard deviation of 0.1 (not reported in the original publication). The overall goodness-of-fit, GOF = 0.77, suggests that the points are scattered by 0.077 log units about the best-fit curve.

NSC-639829 pK$_a$

The two MarvinSketch-predicted pK_as for NSC-639829 were far from what was indicated in Figure 3b. It is quite clear that there is no acidic ionization corresponding to the urea NH group below pH 12. This was also the finding of the spectrophotometric pK_a determination. The dimethylaniline amine S_{pH}-pK_a and the predicted value (Table 1) were different by 2.4 log units. With the adage "prediction guides but experiment decides," it was decided to measure the pK_a(s) of NSC-639829 by an independent method. One pK_a was evident by the spectrophotometric method, and its value was different from both the predicted and the refined S_{pH}-pK_a values (Table 1). In depth analyses of many log *S* – pH profiles [10, 12, 18] show that pK_a values determined by modern purpose-built pK_a instrumentation [13, 14] can differ substantially from S_{pH}-pK$_a$ values, suggesting that the simple Henderson-Hasselbalch equation may not always be an accurate predictor of the pH dependence of solubility. This is particularly evident in examples such as prostaglandin F2α [6]. We consequently pursued possible explanations for the difference between S_{pH}-pK_a = 4.70 ± 0.12 and the UV-measured pK_a = 3.76 ± 0.03.

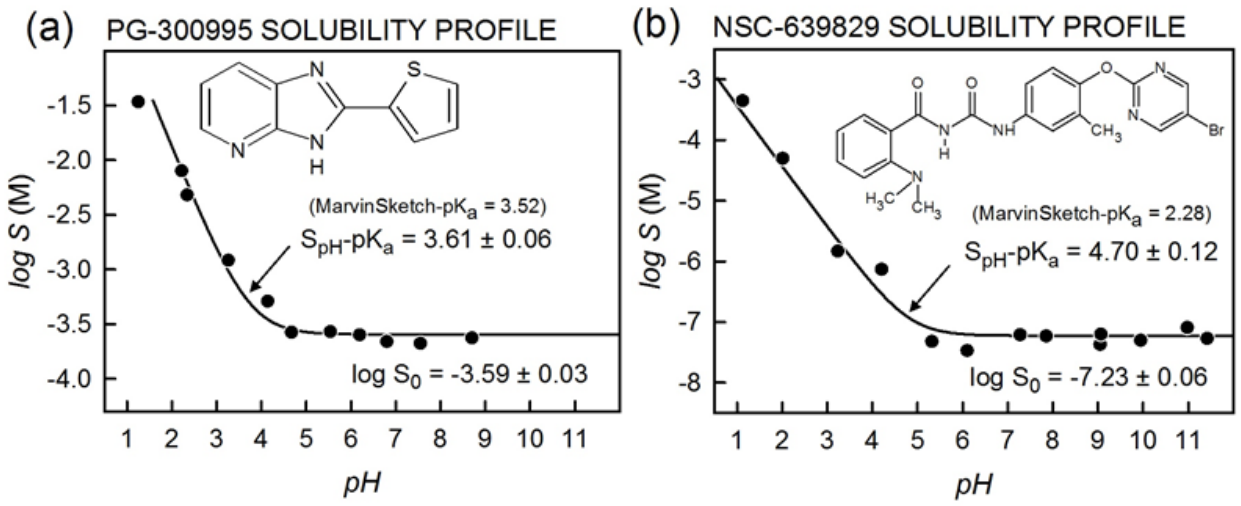

Figure 3. (a) solubility profiles of (a) PG-300995 and **(b)** NSC-639829. The diagonal region has slope of -1. The pH in the bend, between the slope -1 and slope 0 is the apparent pK_a, which may or may not be the true pK_a.

A step-by-step solubility model construction was described recently by Avdeef [10]. Knowing the accurate pK_a starts the process. The shape of the log *S* – pH curve is compared against a series of templates [4,9,10]. For the profile in Figure 3b, the key characteristics are: $pK_a^{app} > pK_a$ and slope = -1 in the diagonal region, suggesting that a mixed-charge dimer (or higher order oligomer) needs to be considered (CASE 3b [4,9,10,12]). The log S_0 can be estimated as the solubility in the pH >> pK_a region of the curve. Three equilibria are needed to describe such a model: $H^+ + B \leftrightarrow BH^+ (pK_a)$, $B \leftrightarrow B(s)$ $(1/S_0)$, and $BH^+ + B \leftrightarrow BHB^+$ (K_2). Having initial estimates of constants corresponding to proposed equilibrium reactions, it is possible to refine the model by weighted nonlinear regression, a procedure described elsewhere [18]. The iterative refinement process continued to convergence.

Figure 4 shows the refined results for NSC-639829. The solid (red) line is the best fit to the log *S* data at various pH values. The dashed line is calculated by the Henderson-Hasselbalch equation, incorporating the spectrophotometrically-measured pK_a.

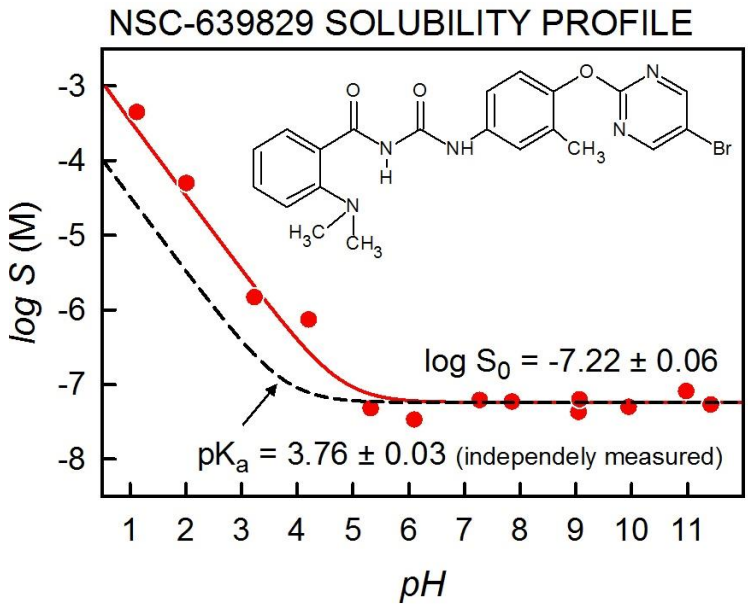

Figure 4. Solubility profile of NSC-639829 incorporating the UV-determined pK_a in the equilibrium model. The dashed line is calculated using the Henderson-Hasselbalch equation.

Figure 5 shows the distribution of various species as a function of pH.

At pH = S_{pH}-pK_a (4.70) in a suspension containing 1 mg/mL added drug, the concentration of the free base, [B] = 5.79 x 10^{-8} M (= S_0, the intrinsic solubility), accounts for 66 % of the total aqueous concentration of NSC-639829. At this pH, [BH^+] accounts for 8 % of the total aqueous concentration of the drug, while [BHB^+] accounts for the remaining 26 %. The molecule is 34 % ionized at this pH.

At pH = pK_a (3.76) in a 1 mg/mL suspension, the concentration of the free base remains the same, but it accounts for only 18 % of the total aqueous concentration of NSC-639829. At this pH, [BH^+] also accounts for 18 % of the total ([B] = [BH^+] when pH = pK_a), while [BHB^+] accounts for the remaining 64 %. The molecule is 82 % ionized at this pH.

According to the equilibrium model, the ratio [BHB^+]/[BH^+] = 3.4 is expected to remain unchanged as long as the solutions remain saturated (as suggested by the two thick diagonal lines of identical slope -1 in Figure 5), provided there are no other unaccounted equilibria when the positively charged species become less concentrated than the uncharged free base (pH > 4.7).

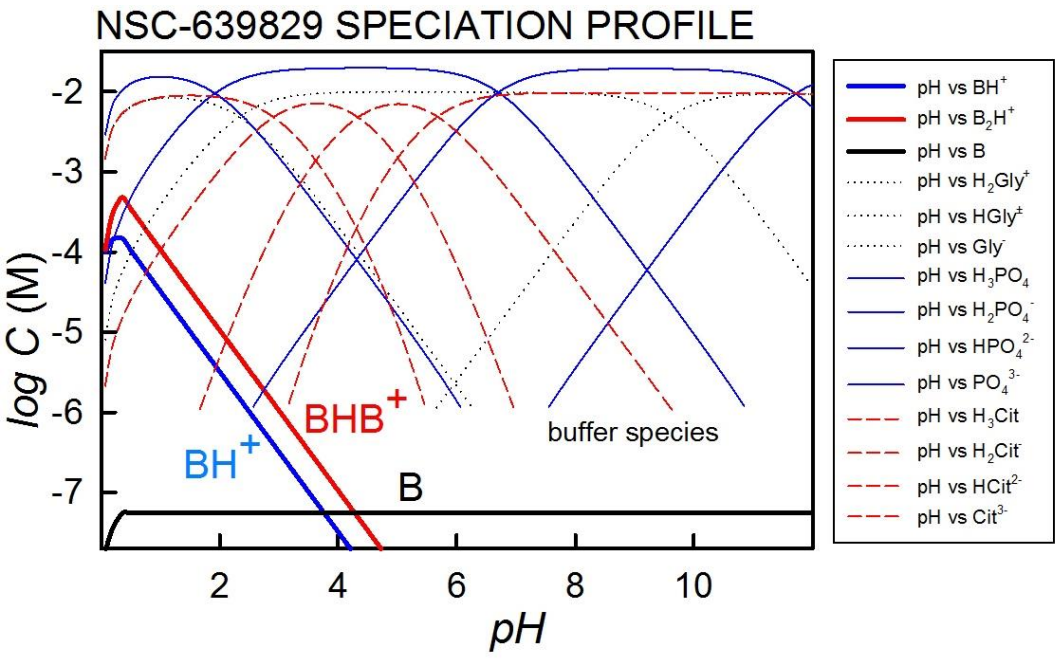

Figure 5. Speciation profile of NSC-639829. The thick solid lines correspond to the concentrations of the drug species. The thin lines correspond to the concentrations of the three buffer components used to simulate the data: glycine, phosphate, and citrate. The 1 mg/mL used in the simulation suggests that all of the solid dissolves when pH < 0.5.

Conclusions

We re-analyzed the previously published solubility-pH data of two weak base drugs. It was tentatively proposed, based on the agreement between the apparent pK$_a$ (S_{pH}-pK_a) and that predicted by MarvinSketch and ADMET Predictor (but not ACD/Percepta), that the solubility profile of the more soluble drug, PG-300995, could be adequately predicted by the Henderson-Hasselbalch (HH) equation. However, the practically-insoluble NSC-639829 drug could not be predicted by the simple HH equation. Its pK_a was determined here. The inclusion of the independently-measured pK_a in the equilibrium model

suggested that the "anomalous" profile of NSC-639829 can be explained by presence of a mixed-charge cationic dimer (BHB^+). This illustrates that an independently-determined accurate pK_a is critical to interpreting solubility-pH data of ionizable compounds. However, predicted pK_a values can be very helpful guides in the initial stages of such investigations. Apparently, the simple Henderson-Hasselbalch equation holds for PG-300995, but not NSC-639829.

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Appendix – Derivation of the Solubility - pH Equations

In the case of a monoprotic weak base, a saturated solution can be defined by the equations and the corresponding constants

$$BH^+ \leftrightarrows H^+ + B \qquad K_a = [H^+][B] / [BH^+] \qquad (A1)$$

$$B(s) \leftrightarrows B \qquad S_0 = [B] \qquad (A2)$$

Solubility, *S*, at a particular pH is defined as the mass balance sum of the concentrations of all of the species dissolved in the aqueous phase:

$$S = [B] + [BH^+] \qquad (A3)$$

where the square brackets denote molar concentration of species. The above equation can be transformed into an expression containing only constants and $[H^+]$ (as the only variable), by substituting the ionization and solubility Eqs. (A1) and (A2) into Eq. (A3).

$$\log S = \log ([B] + [H^+][B] / K_a)$$

$$= \log [B] + \log (1 + [H^+] / K_a)$$

$$= \log S_0 + \log (1 + 10^{+pKa - pH}) \qquad (A4)$$

Eq. (A4) is usually called the Henderson-Hasselbalch equation for a monoprotic weak base, and describes a hyperbolic-shaped log *S* – pH curve. At the bend in the log *S* – pH curve, the pH equals the pK_a.

It can be hypothesized that the dimeric mixed-charge weak base species, $(B.BH^+)$, also forms, which contains a 1:1 ratio of B and BH^+. An additional equilibrium equation needs to be added to the mass balance.

$$B + BH^+ \leftrightarrows (B.BH^+) \qquad K_2 = [B.BH^+] / [B][BH^+] \qquad (A5)$$

Eq. (A3) needs to be expanded accordingly.

$$S = [B] + [BH^+] + 2\,[B.BH^+] \qquad (A6)$$

In logarithmic form,

$$\log S = \log ([B] + [H^+][B] / K_a + 2\,K_2[B][BH^+])$$

$$= \log ([B] + [H^+][B] / K_a + 2\,K_2[B]^2\,[H^+] / K_a)$$

$$= \log S_0 + \log (1 + \{ 1+2\,K_2\,S_0 \}\, 10^{+pKa - pH}) \qquad (A7)$$

For NSC-639829, the factor in the braces in Eq. (A7) equals 8.60 ($=1 + 2 \times 10^{+7.80} \times 10^{-7.22}$). When the logarithm of the factor (0.94) is taken into the exponent, the resulting equation appears to equal Eq. (A4), except that the pK_a is replaced with the S_{pH}-pK_a value (3.76+0.94 = 4.70). The hypothesized presence of the mixed-charge dimer appears to shift the original Henderson-Hasselbalch equation in the positive pH direction by nearly a log unit. The presence of self-aggregated species of other stoichiometries can distort the shape of the simple Henderson-Hasselbalch equation in a number of different ways [4,9,10,12,18]. The example in the present study is one of the simpler types of distortion.

With increasing pH, it is possible for the protonated dimer, $B.BH^+$, to lose the ionizable proton to become the water-soluble neutral-species dimer, B_2. The pK_a for such a process would not be expected to be exactly the same as the measured pK_a. For example, ketoprofen bound to sodium taurocholate

micelles shows such a secondary pK_a [9]. There was no hint of such a process in the case of NSC-639829, but a more thorough investigation would require additional log *S*-pH measurements with total concentration of the sample varied over a wide range, since aggregates would be expected to have concentration dependence.