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Anomalous Solubility Behavior of Several Acidic Drugs

Alex Avdeef

in-ADME Research, 1732 First Avenue, #102, New York, NY 10128, USA
E-mail: alex@in-ADME.com; Tel.: +1 646 678 5713

Abstract

The "anomalous solubility behavior at higher pH values" of several acidic drugs originally studied by Higuchi et al. in 1953 [1], but hitherto not fully rationalized, has been re-analyzed using a novel solubility-pH analysis computer program, pDISOL-X^{TM}. The program internally derives implicit solubility equations, given a set of proposed equilibria and constants (iteratively refined by weighted nonlinear regression), and does not require explicit Henderson-Hasselbalch equations. The re-analyzed original barbital, phenobarbital, oxytetracycline, and sulfathiazole solubility-pH data of Higuchi et al. is consistent with the presence of dimers in saturated solutions. In the case of barbital, phenobarbital and sulfathiazole, anionic dimers, reaching peak concentrations near pH 8. However, oxytetracycline indicated a pronounced tendency to form a cationic dimer, peaking near pH 2. Under the conditions of the original study, only barbital indicated a slight tendency to form a salt precipitate at pH > 6.8, with a highly unusual stoichiometry (consistent with a slope of 0.55 in the log S – pH plot): $K^+ + A_2H^- + 3HA \leftrightarrows KA_5H_4(s)$. Thus the "anomaly" in the Higuchi data can be rationalized by invoking specific aggregated species.

Keywords

Sparingly-soluble drugs; pH-dependent solubility; salt solubility products; solubility equations; aggregation; shake-flask method.

Introduction

Aggregation and micelle formation of drugs in a saturated solution can complicate the interpretation of the solubility measurements, especially when the molecules are ionizable. There are many reports of such systems [1-6]. For example, doxycycline [3], with intrinsic solubility S_0 = 0.72 mg/mL, at 25°C in 1 M NaCl/HCl solutions (pH 0 to 6), forms dimers with K_2 = 24 M^{-1}. Such aggregates were found to have unusually high and temperature-sensitive solubility. Zhu and Streng [4] found that the self-association of dolasetron to form cationic dimers and trimers was enthalpy driven (H-bond/aromatic ring rather than hydrophobic/electrostatic interactions), with the aggregation constants, K_{2-3}, ranging from 4 to 50 M^{-1} at 25°C. Many nonsteroidal antiinflammatory drugs [5], such as indomethacin, diclofenac, ibuprofen, ketoprofen, naproxen, and sulindac, can self-associate by forming mixed-charge micelle or micelle-like structures. The reported CMC of diclofenac is about 25 mM and of ketoprofen is about 160 mM [5]. The solubility-pH profiles cannot be accurately described with the Henderson-Hasselbalch equation when aggregates form [2,6].

The objective of this brief commentary is to address the "anomalous solubility behavior at higher pH values" of several ionizable drugs studied by Higuchi et al. in 1953 [1], but hitherto not fully understood. The measured solubility-pH profiles of two barbiturates (phenobarbital, barbital) and two ampholytes (oxytetracycline and sulfathiazole) showed anomalous shapes, which could not be accurately described by the Henderson-Hasselbalch equations. Analysis of the data using a new program, *p*DISOL-X, suggested the presence of anionic (mixed-charge) dimers, AHA^-, in the case of the barbiturates and sulfathiazole and very stable cationic (mixed-charge) dimers, $XH_2 \cdot XH_3^+$, in the case of oxytetracycline. Higuchi et al. noted that between pH 6.8 and 7.1, the slope in the log S vs. pH plot for barbital had a value of +0.55, but no concrete explanation was offered. They also noted the oxytetracycline between pH 2 and 5 had a slope of -0.91, which would not have been expected for a simple system. The values of the pK_a of the molecules were not reported in the study. Had accurate pK_a values been discussed, then other anomalies in the solubility data would have been evident at that time.

Experimental

Legacy Data

In the Higuchi et al. study [1], pH was adjusted by the addition of standardized HCl or KOH to buffer-free suspensions containing 10-30 % excess solid over the saturation concentration. The ionic strength was well controlled with 0.15 M KCl, and the temperature was maintained at 25 °C (30 °C in one case). The equilibration times ranged from one to eight days, after which the final pH had been measured.

Refinement of Intrinsic and Salt Solubility and Aggregation Constants

Detail of the mathematical approach in the *p*DISOL-X computer program has been described by Völgyi et al. [6]. Briefly, the new data analysis method uses log S - pH as measured input data from any analytical technique (along with standard deviations, SD(log S)) into *p*DISOL-X. An algorithm was developed which considers the contribution of all species proposed to be present in solution, including universal buffer components (e.g., Britton-Robinson). 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 [2,6]. Therefore, in principal, drug-salt precipitates, -aggregates, -complexes, - bile salts, -surfactant can be accommodated [2,6]. Presence of specific buffer-drug species can be tested. The program assumes an initial condition of a suspension of the solid drug in a solution, ideally with the suspension saturated over a wide range of pH. The program calculates the distribution of species consequent to a sequence of additions of standardized strong-acid titrant HCl (or weak-acid titrants H_3PO_4, H_2SO_4, acetic acid, maleic acid, lactic acid) to simulate the suspension pH-speciation down to pH ~ 0, the staging point for the subsequent operation. A sequence of perturbations with standardized KOH 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 [6].

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 minimum, as described elsewhere in detail [2,6].

Results and Discussion

The results of the analysis of the Higuchi et al. data are summarized in Table 1. All four drugs in the Higuchi study indicated the presence of singly-charged dimers in saturated solution of the free acid. A salt precipitate also formed in the case of barbital.

Table 1. Summary of the Results of the Re-Analysis of the Higuchi et al. [1] Data.

COMPOUND	pK_a (25°C, I =0.15 M) [a]	-log S_0	S_0 (mg/mL)	mixed-charge dimer	log K_2 (M^{-1})	K_2 (M^{-1})	GOF [b]
Barbital [c]	7.86	1.57 ± 0.01	5.00	HA+A$^-$ ⇄ A$_2$H$^-$	2.17 ± 0.03	148 ± 10	0.12
Phenobarbital	7.41	2.55 ± 0.01	0.66	HA+A$^-$ ⇄ A$_2$H$^-$	2.43 ± 0.04	269 ± 22	0.22
Oxytetracycline	8.82, 7.22, 3.23	3.20 ± 0.03	0.29	XH$_3^+$+XH$_2$ ⇄ X$_2$H$_5^+$	3.42 ± 0.07	2634 ± 425	0.75
Sulfathiazole	7.12, 2.30	2.83 ± 0.01	0.37	HX+X$^-$ ⇄ X$_2$H$^-$	1.50 ± 0.10	32 ± 7	0.12

[a] pK_a values taken from the "Wiki-pKa" database: <http://www.in-adme.com/wiki_pka.php/>.
[b] GOF = goodness-of-fit, based on assumed individual measurement errors of 0.1 log units.
[c] 30°C. The other three molecules were studied at 25°C.

Barbital

The solid curve in Figure 1a is the log S vs. pH profile of barbital at 30 °C. The dashed curve in the figure was calculated using the Henderson-Hasselbalch equation, employing pK_a of 7.86. As can be seen, the latter equation does not predict the experimental points very well.

Figure 1b shows the simulated titration curve (volume of 1 M KOH added per mL of suspension solution vs. pH) for barbital that best describes the Higuchi data (discrete points). The amount of solid barbital added to 1 mL of water was taken as 250 mg in the *p*DISOL-X data analysis. The flat region centered at pH 6.82, bounded by two discontinuities, is due to the co-precipitates of free acid, HA(s), and complex salt, KA$_5$H$_4$(s), i.e., [K$^+$(HA)$_3$AHA$^-$](s). This fixed-pH region is defined by the so called pK_a^{GIBBS} [7,8]. The free acid is the only precipitate present for pH < pK_a^{GIBBS}. It takes about 0.3 mL KOH titrant to convert the free acid precipitate entirely into the salt form, as shown in Figure 1b. For pH > pK_a^{GIBBS}, KA$_5$H$_4$(s) is the sole precipitate, up to the peak pH 9.56, where the salt precipitate completely dissolves due to ionization leading to the formation of the anion, A$^-$ (Fig. 1d). Note that the width of the flat region of the titration curve (Fig. 1b), and hence the values of the pK_a^{GIBBS} and peak pH are conditional, since the extent of dimer formation depends on the amount of solid added to water. Under the conditions of the study, only barbital appeared to show a tendency to form a salt precipitate, consistent with the very unusual stoichiometry (slope of +0.55 in the log S-pH plot): KA$_5$H$_4$(s) ⇄ K$^+$ + AHA$^-$ + 3HA, with K_{sp} = 2.23 x10^{-8} M^5.

The above salt stoichiometry best rationalizes the log S − pH curve of barbital for pH > pK_a^{GIBBS}. The solubility curve (solid line) for pH < pK_a^{GIBBS} (6.82*)* is horizontally displaced to lower pH by about one unit, without change in its *shape*, compared to that calculated using the Henderson-Hasselbalch equation (dashed line in Fig. 1a). This is consistent with the formation of a mixed-charge dimeric species, AHA$^-$ (in the saturated solution), which may be visualized as a conjugate of a free acid, HA, and the ionize species, A$^-$ (cf., Appendix). The AHA$^-$ dominates in concentration over that of A$^-$ up to pH 9 (Fig. 1c). For pH > pK_a^{GIBBS}, the log S curve takes on a slope of about +0.55, as originally noted by Higuchi et al. This can be explained if a new precipitate replaces HA(s). A reasonable choice might have been K$^+$·AHA$^-$(s), since AHA$^-$ is the dominant anion present in solution. However, the log S curve is not well fitted with such a model. Further testing of the data could have assumed that the above salt precipitate also contained HA components.

After a trial-and-error process, the best fit was found where three molecules of the free acid, HA, and one molecule of AHA⁻ combine with K⁺ to form the putative solid with the unusual stoichiometry $K^+(HA)_3AHA^-$(s). *p*DISOL-X can easily consider new tentative species in the model development and quickly test the data against such species, without having to derive explicit new solubility equations. The method described in the Appendix A in [6] certainly could have been applied here to derive the explicit equation corresponding to the formation of $K^+(HA)_3\,AHA^-$(s), but the resultant equation would have been quite complex and of limited general applicability.

Figure 1c shows the direct concentration of various barbital species as a function of pH, while Figure 1d illustrates the log concentration vs pH relationships. In alkaline solution, the monomeric species, A⁻, and the mixed-charge dimeric species, AHA⁻ coexist, with the latter species reaching a maximum concentration at the peak pH 9.56 (Fig. 1c) and declining in concentration with further increases in pH.
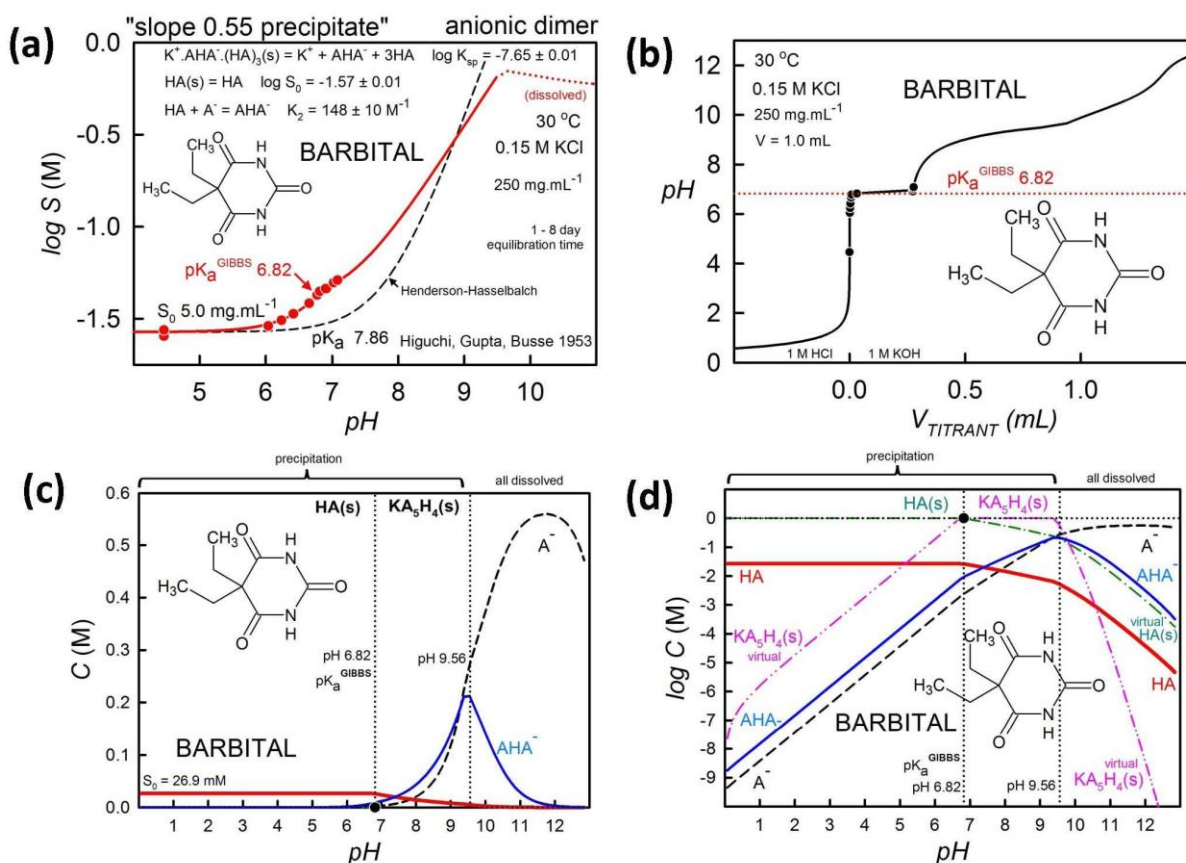
Figure 1. Barbital: **(a)** log *S* vs. pH; **(b)** titration curve; **(c)** concentration of species as a function of pH; **(d)** log concentration of species as a function of pH.

The above case study of barbital is an example of a general methodology to rationalize complicated log *S* - pH profiles. In a simple "Henderson-Hasselbalch" precipitation process, the log *S* curve does not depend on the quantity of solid added, as long as enough solid is added to maintain a saturated solution. However, if an aggregate, oligomer, or micelle-like drug forms, then the measured solubility can critically depend on the amount of solid added to solution. For example, suppose a micelle of the drug forms above the critical micelle concentration (CMC). A very poorly soluble drug can become better (self-) solubilized in high concentration (>CMC). For example, this could explain why a parenteral formulation of amiodarone (high concentration) can stop atrial fibrillation in a few minutes post injection, whereas an oral formulation may take 30 days to reach full therapeutic effectiveness [9]. Being able to quantitate such concentration dependence (due to aggregate formation, etc.) could be a practically useful feature of *p*DISOL-X in drug

development, to help predict dosing effects of poorly soluble molecules which form aggregates in solution.

Phenobarbital

Phenobarbital, which is about eight times less soluble than barbital, shows a similar solubility-pH profile in Figure 2a as that of barbital, except that no salt precipitate forms (the ratio of the octanol-water partition coefficients of the two molecules is also about eight). A dimeric species of the same stoichiometry forms as in the case of barbital, reaching maximum concentration at (conditional) pH of 7.82, as shown in Figure 2b (the pH-speciation simulation assumed the 5 mg of solid had been added to 1 mL of water). The presence of the dimer is indicated by the shift of the log S – pH profile to the lower pH values above pH 5, compared to that predicted by the Henderson-Hasselbalch equation.
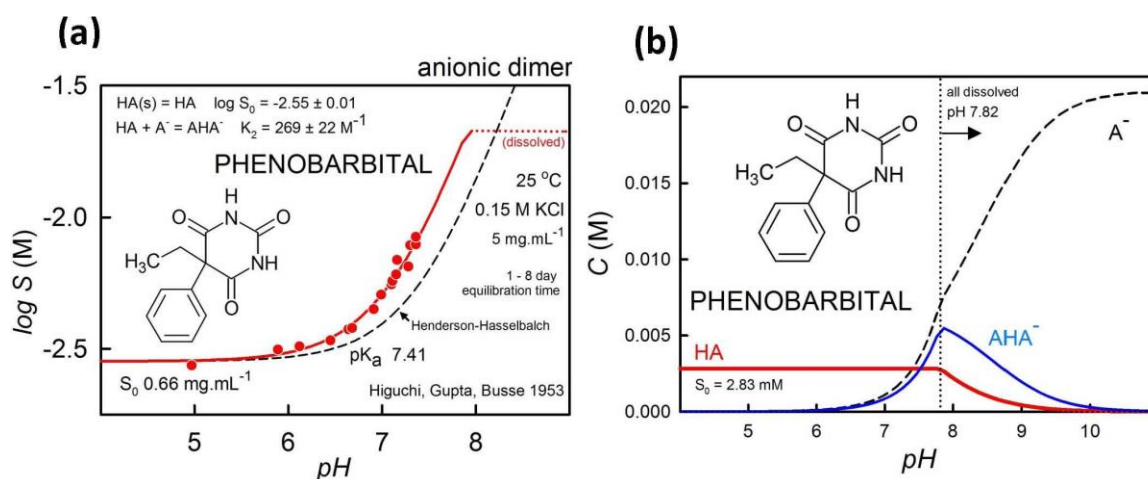

Figure 2. Phenobarbital: **(a)** log S vs. pH; **(b)** concentration of species as a function of pH.

Oxytetracycline

Oxytetracycline is an ampholyte, whose ionizations can be described by three pK_a values (Table 1). Figure 3a shows the solubility-pH curve shifted to higher pH values below pH 5, compared to what is predicted by the Henderson-Hasselbalch equation. This is a pattern consistent with the presence of a cationic dimer formed from the neutral XH_2 species and the cation XH_3^+. Many other shift patterns have been described in reference [2]. The cationic dimer maximized in concentration at (conditional) pH 1.63, as shown in Figure 3b (the pH-speciation simulation assumed that 50 mg of solid had been added to 1 mL of water).
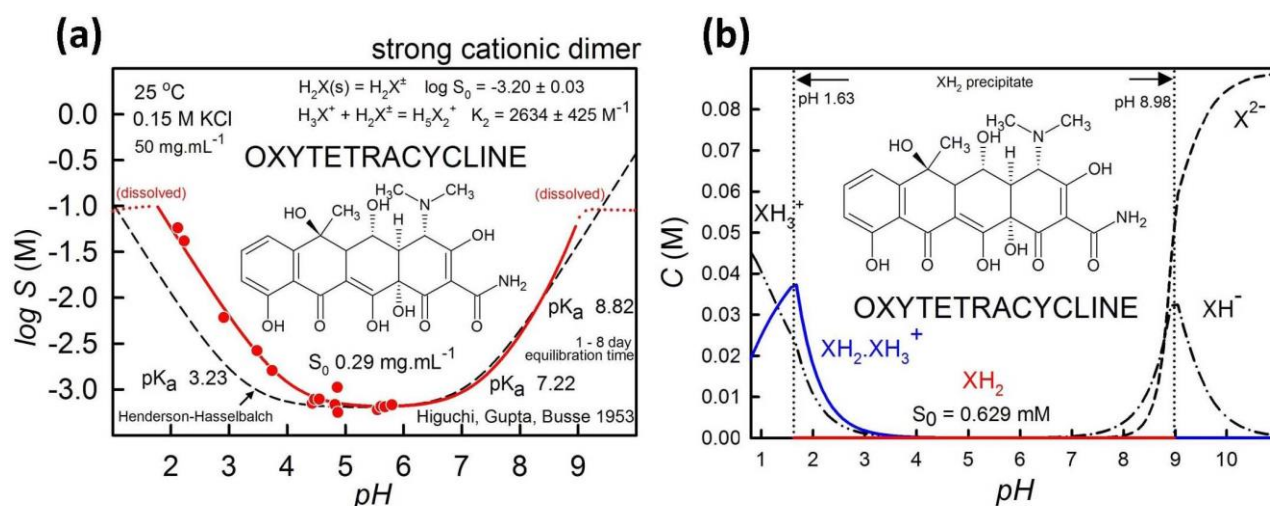
Figure 3. Oxytetracycline: **(a)** log *S* vs. pH; **(b)** concentration of species as a function of pH.

Sulfathiazole

The ampholyte sulfathiazole has the least tendency to form dimers, as suggested in Figure 4. A small quantity of anionic dimer appears to be consistent with the high precision data, whose concentration reaches a maximum at pH 8.16. Invoking the presence of a dimeric species under the circumstance would not be warranted ordinarily, were it not for the exceptionally high precision of the Higuchi et al. data.
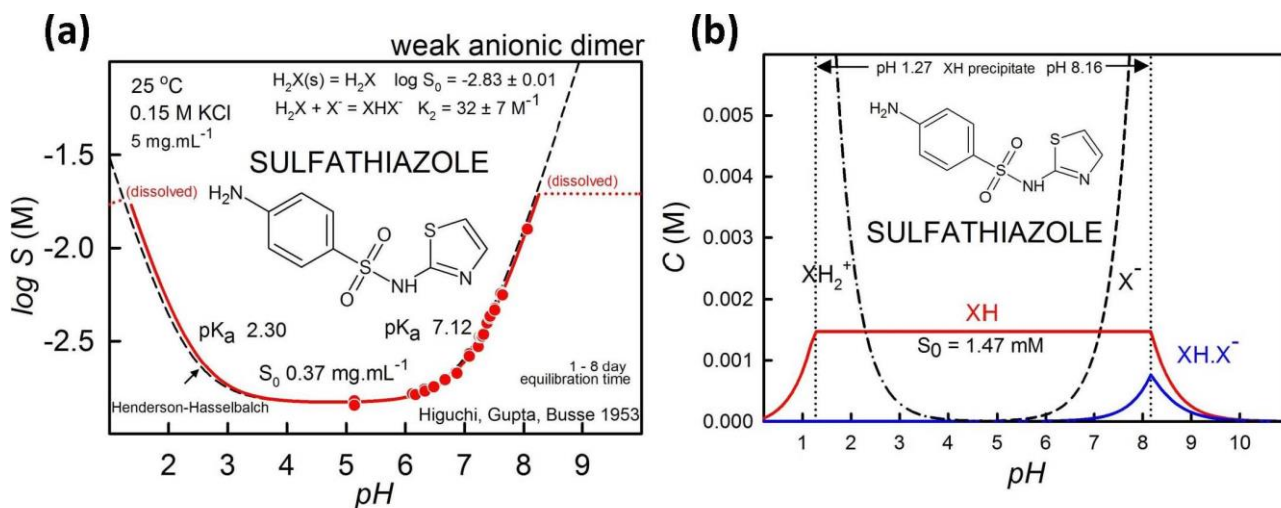

Figure 4. Sulfathiazole: **(a)** log *S* vs. pH; **(b)** concentration of species as a function of pH.

Conclusions

In the *p*DISOL-X analysis, the "anomaly" in the Higuchi et al. 1953 data could be explained by presence of anionic dimers in three cases, and by the strong presence of a cationic dimer in the case of oxytetracycline, and also by the barbital salt precipitate that exhibits the slope of +0.55. Apparently, these species were either not recognized originally, or were not fully characterized. Such anomalies can be common with sparingly-soluble drugs, but are not always easy to recognize. *p*DISOL-X could be a helpful new tool to further aid in the analysis of dissolution mechanisms of sparingly-soluble drugs, since dissolution is closely linked to solubility.

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Appendix

Shape Pattern Interpretation in Solubility Profiles for Sparingly-Soluble Ionizable Weak Acids

An important part of the analysis of solubility profiles involves recognizing characteristic shapes and interpreting these in terms of an equilibrium model [2]. Figure 5 shows the results of a series of simulations of a weak acid, MW 500 g/mol, having the same pK_a = 7 and pS_0 = 3, but having different combinations of salt and aggregate formations. In the first frame, 50 mg of solid free acid were added to 1 mL of 0.15 M KCl solution. In the rest of the frames, 1500 mg were added. The calculated average ionic strength is indicated in red at the bottom right of each frame. The dashed curves in the figures are calculated from the Henderson-Hasselbalch equation using the pK_a and pS_0 constants. The solid lines are the actual log S curves, where either the free acid or the salt is precipitated. The pH at which both the free acid and the salt co-precipitate is the pK_a^{GIBBS} (Figs. 5b-d). In cases where pH is raised past the point where the entire solid dissolves, the curve is indicated by a dotted line (Figs. 5a,e,f).
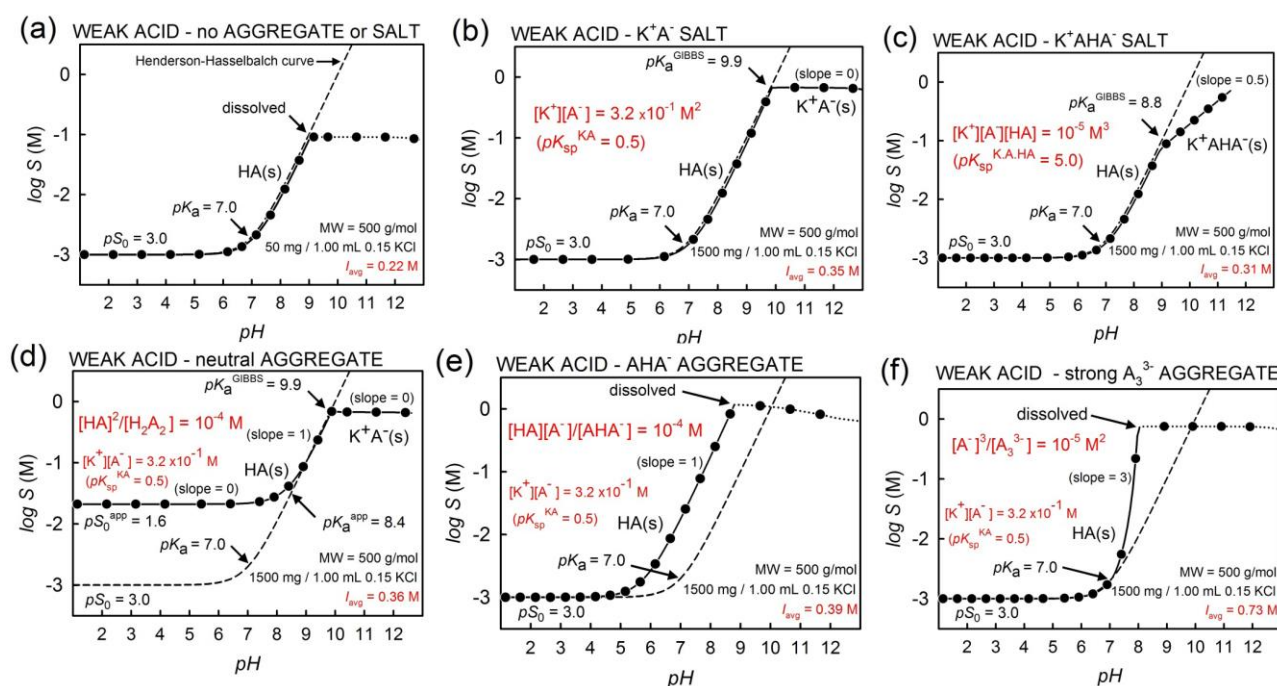
Figure 5. Simulations of various cases of free acid log *S* – pH curves where salt precipitates or where aggregates form (see text).

Figure 5a is the simplest case, with no salt precipitate and no aggregates. The Henderson-Hasselbalch equation describes the log *S* – pH profile as long as the solution remains saturated. Figures 5b-d show the shape of the profiles as K^+A^-(s) or K^+AHA^-(s) salts precipitate for pH ≥ pK_a^{GIBBS}. Notice that in Figure 5c, the maximum solubility does not occur at pH = pK_a^{GIBBS}. In older literature, it had been popular to refer to pK_a^{GIBBS} as "pH_{max}", but clearly, such nomenclature is misleading in some cases, such as when complex salts form, as in Figure 5c [7,8]. In Figures 5d-f cases, aggregates form, which elevates solubility in characteristic ways. In the case of neutral aggregates (Fig. 5d), the region of the intrinsic solubility (pH < pK_a) is raised due to the enhanced solubility of the neutral species. The apparent pK_a is shifted to 8.4 from the true value of 7. In the cases of charged aggregates (Fig. 5e,f), the solubility product for K^+A^-(s) is no longer exceeded (for 1500 mg/mL), and the compound completely dissolves above about pH 8, due to the increased solubility in alkaline solution. Figure 5e is an example of a mixed-charge dimer formation, AHA^-, where the solubility

curve in the slope = 1 region shifts to lower pH values compared to that predicted by the Henderson-Hasselbalch equation. In Figure 5f, an anionic trimer, A_3^{3-}, is indicated by the slope = 3 in the diagonal region of the log S profile.

Example of a Step by Step Model Construction and Refinement for Ionizable Sparingly-Soluble Weak Acid

The following is a brief description of a practical process for developing and refining an equilibrium model to describe the solubility behavior of sparingly soluble drugs.

1. Before starting to develop an equilibrium model for an ionizable molecule, it is first necessary to know the accurate value of the pK_a. Preferably, the pK_a is determined by an independent technique (e.g., by potentiometry, spectrophotometry, or capillary electrophoresis – but *not* by solubility).

2. Plot the log *S* data as a function of pH. With an accurate pK_a value at hand, the log *S* profile is inspected for its shape characteristics, to see if the profile resembles any of the template frames in Figure 5.

3. If the pK_a appears to be *higher* from the true value (as indicated by the pH in the middle of the bend in the log *S* curve), then either neutral aggregates form (Fig. 5d) or not enough time had been allowed for equilibrium to be reached. The pS_0 can be estimated (for acids) as $pS_0^{app} + (pK_a^{app} - pK_a)$ [2]. Select $H^+ + A^- \leftrightarrows HA$, $HA \leftrightarrows HA(s)$, and $2HA \leftrightarrows A_2H_2$ as the three equilibria to consider in the model, and associate pK_a and pS_0 with the first two equations. Take a guess on the third constant, e.g., assume it to be 3 (log units). Refinement will usually hone in on the appropriate value quickly enough if the equilibrium model is valid.

4. If the pK_a appears to be *lower* than the true value, but the slope in the diagonal portion of the log *S* profile is 1, then a mixed-charge dimer (or higher order oligomer) may be present (cf., Fig 5e). The pS_0 can be estimated as solubility in the acidic region of the curve (pH << pK_a). Select $H^+ + A^- \leftrightarrows HA$, $HA \leftrightarrows HA(s)$, and $HA + A^- \leftrightarrows AHA^-$ as the three equilibria to consider in the model, and associate pK_a and pS_0 with the first two equations. Take a guess on the third constant, e.g., assume it to be 3 (log units).

5. If the pK_a appears to be *slightly lower* than the true value, but the slope in the diagonal portion of the log S profile is greater than 1, then an anionic oligomer may be present, with the degree of aggregation equal to the value of the slope (cf., Fig 5f). The pS_0 can be estimated as solubility in the acidic region of the curve (pH << pK_a). Select $H^+ + A^- \leftrightarrows HA$, $HA \leftrightarrows HA(s)$, and $nA^- \leftrightarrows A_n^{n-}$ as the three equilibria to consider in the model, and associate pK_a and pS_0 with the first two equations. Take a guess on the third constant, e.g., assume it to be 3 (log units).

6. It is possible that more than one mechanism is associated with the solubility profile. Therefore, a combination of the above steps may be needed.

7. If the curve levels off in the alkaline solution (pH >> pK_a), as in Figure 5, then a salt may have precipitated in alkaline solution. It needs to be ascertained that the effect is not due to the complete dissolution of solid, as in Figures 5a,e,f. Enter the appropriate equilibrium expression along with a guess of the value of the constant.

8. Having initial estimates of constants corresponding to proposed equilibrium reactions, the model is ready for weighted nonlinear regression, a procedure described elsewhere [2,6]. The iterative refinement will test the consistency between the data and the model. If the agreement is not good

(root-mean square error >> 0.1), then the model may be modified and the refinement process repeated, until no further improvements are evident.