

Original scientific paper

Salt Solubility Products of Diprenorphine Hydrochloride, Codeine and Lidocaine Hydrochlorides and Phosphates – Novel Method of Data Analysis Not Dependent on Explicit Solubility Equations

Gergely Völgyi¹, Attila Marosi¹, Krisztina Takács-Novák¹, Alex Avdeef^{2*}

¹Semmelweis University, Department of Pharmaceutical Chemistry
H-1092 Hőgyes Endre street 9. Budapest, Hungary

²in-ADME Research, 1732 First Avenue, #102, New York, NY 10128, USA

*Corresponding Author: Alex Avdeef;

E-mail: alex@in-ADME.com;

Tel.: +1 647 678 5713;

Received: December 6, 2013; Revised: December 11, 2013; Published: December 16, 2013

Abstract

A novel general approach was described to address many of the challenges of salt solubility determination of drug substances, with data processing and refinement of equilibrium constants encoded in the computer program pDISOL-XTM. The new approach was illustrated by the determinations of the solubility products of diprenorphine hydrochloride, codeine hydrochloride and phosphate, lidocaine hydrochloride and phosphate at 25 °C, using a recently-optimized saturation shake-flask protocol. The effects of different buffers (Britton-Robinson universal and Sørensen phosphate) were compared. Lidocaine precipitates were characterized by X-ray powder diffraction (XRPD) and polarization light microscopy. The ionic strength in the studied systems ranged from 0.25 to 4.3 M. Codeine (and possibly diprenorphine) chloride were less soluble than the phosphates for pH > 2. The reverse trend was evident with lidocaine. Diprenorphine saturated solutions showed departure from the predictions of the Henderson-Hasselbalch equation in alkaline (pH > 9) solutions, consistent with the formation of a mixed-charge anionic dimer.

Keywords

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

Introduction

Salt selection in preformulation is an important step in the preparation of effective oral drug formulations [1-8]. Salt solubility, being a conditional constant, takes on different values according to the concentrations and types of reactants used in a particular study. For this reason, laboratory-to-laboratory comparisons can be complicated, possibly leading to conflicting interpretations of in vitro dissolution studies in formulation development. On the other hand, salt solubility products are true equilibrium constants. But these are not often reported in published salt solubility studies. Interpretation and scaling of salt solubility measurements can be very challenging for a number of reasons.

- Since drug salts are often much more soluble than the corresponding uncharged forms, salt solubility measurement is usually carried out in relatively concentrated solutions, with ionic strengths, I , often exceeding 1 M.
- At these high levels, activity coefficients of ions are poorly controlled and cannot be accurately predicted by the traditional Debye-Hückel equation.
- Also, pH electrodes calibrated in buffers with $I = 0.15$ M (“physiological” level) may not be accurate at much higher ionic strengths, especially in the extreme regions of pH (< 1 or > 12) where electrode junction potentials may be very different from those characteristic of the physiological level.
- The salt solubility is a conditional constant, which depends not only on the concentration of the drug but also on that of the counterion with which the charged drug precipitates. The counterion may originate from the buffer used or other unsuspecting solution additives.
- Under such high sample concentrations, many drugs, especially those likely to be surface-active, can form micelles, self-associated aggregates (dimers, trimers, or higher-order oligomers), or complexes with buffer species or other solution additives.

All these effects can complicate the interpretation of the solubility data [9-18]. Such complexity may not be evident unless a full solubility – pH profile is measured, over a pH range containing the uncharged and charged forms of the drug, preferably at more than one solid-sample excess. Carefully planned experimental designs and complicated computations are often needed to correctly interpret the measured salt solubility.

Although explicit solubility equations (cf., Appendix A) have been derived for many different cases of salt solubility and aggregation [19], it is hardly practical to derive such equations for the vast number of possible forms of salt and aggregation stoichiometry that can be encountered. For this reason, salt solubility analysis of data in the past had been done on a case by case basis, sometimes using incomplete explicit solubility equations. At times the impact of aggregation reactions had been recognized but not dealt with quantitatively, presumably because computational methods were not available at the time [9,10,16].

In this study we describe a novel general approach to address many of the challenges of salt solubility determination, with data processing and refinement of equilibrium constants encoded in the computer program pDISOL-XTM (in-ADME Research). The new approach was illustrated by the determinations of the solubility products of three model drugs: diprenorphine hydrochloride, codeine hydrochloride and phosphate, lidocaine hydrochloride and phosphate. Diprenorphine, whose solubility-pH profile had not been reported, is primarily used as an opioid antagonist to reverse the effects of etorphine and carfentanyl. Codeine is a naturally-occurring analgesic opiate. Lidocaine is a local anesthetic. The effects of different buffers (Britton-Robinson universal and Sørensen phosphate) were compared. Lidocaine precipitates were characterized by X-ray powder diffraction and polarization light microscopy. The ionic strength in the studied systems ranged from 0.25 to 4.3 M.

Experimental

Chemicals

Codeine hydrochloride, codeine phosphate, lidocaine free base and lidocaine hydrochloride were purchased from Sigma and used without further purification. Diprenorphine hydrochloride was an in-house synthesized compound and was of analytical grade.

Potassium hydroxide and hydrochloric acid used for pK_a determination were from Sigma. The KOH titrant solution was standardized by titration against primary standards potassium hydrogen phthalate dissolved in 15 mL of 0.15 M potassium chloride. Potassium hydrogen phthalate and potassium chloride were of analytical grade and were purchased from Sigma. The HCl titrant solution was standardized by titrating a measured volume against the standardized KOH.

Two buffer solutions (Britton–Robinson universal and Sörensen phosphate) were used at pH 5 in the shake-flask experiments of codeine and lidocaine. Britton-Robinson buffer solution (mixture of acetic, phosphoric and boric acids, each at 0.04 M) was treated with 0.2 M NaOH to give the required pH. The Sörensen phosphate buffer solutions were prepared by mixing 0.067 M Na_2HPO_4 and 0.067 M KH_2PO_4 solutions to reach the required pH (4.8 – 8.5). Phosphate-containing solutions between pH 1 and 12.5 were used for the equilibrium solubility measurements of diprenorphine, where 0.5 M KOH, 0.5 M or 14.85 M H_3PO_4 (standardized 85%) solutions were used to reach the desired pH values. Distilled water of Ph. Eur. grade was used. All other reagents were of analytical grade.

pH Electrode Standardization and Compensation for Large Changes in Ionic Strength

All of the equilibrium constants reported here are based on the concentration scale, i.e., the “constant ionic medium” thermodynamic standard state [19]. Since the measured pH is based on the operational activity scale, these values need to be converted to the concentration scale, $p_c\text{H}$ ($=-\log[\text{H}^+]$). The procedure to calibrate and standardize the pH electrode is described in detail elsewhere [19]. Briefly, the electrode is standardized by the blank titration method: to a 0.15 M KCl solution, enough standardized 0.5 M HCl is added to lower the pH to 1.8 (0.87 ± 0.03 mL when using a 20 mL solution); then the acidified solution is precisely titrated with standardized 0.5 M KOH up to about pH 12.2 (which consumes about 2 mL 0.5 M KOH). The blank titration pH data are fit to a four-parameter equation [20]:

$$\text{pH} = \alpha + k_s p_c\text{H} + j_H [\text{H}^+] + j_{\text{OH}} K_w/[\text{H}^+] \quad (1)$$

where K_w is the ionization constant of water. The j_H term corrects pH readings for the nonlinear pH response due to liquid junction and asymmetry potentials in highly acidic solutions ($\text{pH} < 2$), while the j_{OH} term corrects for high-pH nonlinear effects [19]. Typical values of the adjustable parameters at 25°C and 0.15 M ionic strength are $\alpha = 0.09$, $k_s = 1.002$, $j_H = 0.5$ and $j_{\text{OH}} = -0.5$. However, each electrode indicates its own characteristic set.

Since salt solubility measurements can be under conditions where ionic strength may reach values as high as 1 M, the experimentally-determined parameters in Eq. (1), based on the “reference” ionic strength 0.15 M, are automatically compensated by the computational procedure in *pDISOL-X*, for changes in ionic strength from the reference value 0.15 M to the actual values in a particular solubility assay, according to the empirically-determined relationships defined elsewhere [19].

pK_a Determination

The pK_a values of diprenorphine were determined by potentiometry using a GLpKa instrument (Sirius, Forest Row, UK) equipped with a combination Ag/AgCl pH electrode. The titrations were carried out at constant ionic strength ($I = 0.15$ M KCl) and temperature ($T = 25.0 \pm 0.5$ °C), and under nitrogen atmosphere. Aqueous solutions of diprenorphine hydrochloride (10 mL, 0.7-0.8 mM) were pre-acidified to

pH 2 with 0.5 M HCl, and then titrated with 0.5 M KOH to pH 12. Three parallel measurements were carried out. The pK_a values were calculated by the RefinementProTM software (Sirius, Forest Row, UK).

Solubility Measurements by Saturation Shake-flask Method

In the shake-flask experiments, the pH of the solutions was measured by a Radiometer PHM 220 pH meter with combined Ag/AgCl glass electrode. The temperature of the samples was maintained at 25.0 ± 0.5 °C during the solubility measurements using a Lauda thermostat. A Heidolph MR 1000 magnetic stirrer was used to mix the two phases. The concentration in the supernatant was measured by spectroscopy using a Jasco V-550 UV/VIS spectrophotometer.

To facilitate the measure of concentration by UV spectrophotometry, the specific absorbance ($A_{1\text{cm}}^{1\%}$, the absorbance of 1 g/100 mL solution over a 1 cm optical path length at a given wavelength) of each sample at the given pH values was determined separately at a selected wavelength using 12-18 points of a minimum of two parallel dilution series, from the linear regression equation (Lambert-Beer law).

The equilibrium solubility of the samples at different pH values was determined by the new protocol of saturation shake-flask method [21]. The sample was added to the aqueous Britton-Robinson universal and Sørensen phosphate buffers (codeine and lidocaine) or phosphate-containing solution (diprenorphine) until a heterogeneous system (solid sample and liquid) was obtained. The solution containing solid excess of the sample was stirred for a period of 6 hours (saturation time) at controlled temperature allowing it to achieve thermodynamic equilibrium. After a further 18 hours of sedimentation, the concentration of the saturated solution was measured by UV spectroscopy. Three aliquots were taken out with a fine pipette (5-500 μL) from the liquid and diluted with the solvent if necessary. At least three parallel concentration measurements were carried out and the result was calculated using 9-12 data points. The standard deviation varied from 1-13%.

Refinement of Intrinsic and Salt Solubility and Aggregation Constants

The new data analysis method uses $\log S$ - pH as measured input data (along with the standard deviations in $\log S$) into the computer program. The data can be UV-derived, potentiometric-derived, etc. An algorithm was developed which considers the contributions of all species present in solution, including universal buffer components (e.g., Britton-Robinson, Sørensen, Prideaux-Ward, etc.). 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. So, in principal, drug-salt precipitates, -aggregates, -complexes, -bile salts, -surfactant can be accommodated [19]. Specific buffer-drug formed species can be tested, as is often necessary with phosphate-, and sometimes, citrate-containing buffers. The program assumes an initial condition of a suspension of the solid drug in a solution often containing a background electrolyte, e.g., 0.15 M NaCl, ideally with the suspension saturated over a wide range of pH. The computer program calculates the distribution of species corresponding 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 speciation down to $\text{pH} \sim 0$, the staging point for the next operation. A sequence of perturbations with standardized NaOH (or KOH) is simulated, and solubility calculated at each point (in pH steps of 0.005-0.2), until $\text{pH} \sim 13$ is reached. The ionic strength is rigorously calculated at each step (cf, Appendix B), and pK_a values (as well as solubility products, aggregation and complexation constants) are accordingly adjusted (cf., Appendix

B). Nonlinear pH electrode standardization parameters (Eq. 1) are included in the calculation [20], a feature that is especially important for measurement of accurate pH in the extreme pH (<1 or >12) regions.

At the end of the speciation simulation, the calculated logS vs. pH curve is compared to actual measured logS vs. pH. A logS-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 logS values reach a minimum. Specifically, the logS - pH data are refined to minimize the weighted residual function,

$$R_w = \sum_i^N \frac{(\log S_i^{obs} - \log S_i^{calc})^2}{\sigma_i^2(\log S)} \quad (2)$$

where N is the measured number of solubility values used to test the model, and $\log S_i^{calc}$ is the calculated log solubility values. The estimated standard deviation in the observed logS, σ_i , is estimated as 0.10 (log units), or is set equal to the values reported in the measurement. The overall quality of the refinement is assessed by the "goodness-of-fit" (GOF),

$$GOF = \sqrt{\frac{R_w}{N - N_p}} \quad (3)$$

where N_p is the number of refined parameters. If a proposed model fits the data well, and accurate weighting factors are used, then $GOF = 1$ is the statistically expected value.

Results and Discussion

Table 1 summarizes some of the physicochemical properties of the three model compounds, including the pK_a constants of diprenorphine determined in this work. Table 2 lists the shake-flask solubility measurement details, including the actual weights of compounds added to form the saturated solutions. In critical salt solubility studies, it is often necessary to state the actual weight of compound added, and not just state that "excess solid was added." This is because salt solubility constants are conditional, and in some cases require the actual weights to determine solubility products, especially when aggregates form in saturated solutions or when salt stoichiometries are complex. In Table 2, the calculated volumes of 14.85 M H_3PO_4 or 0.5 M KOH titrants used to adjust the pH are *normalized* to 1 mL total solution volumes (actual volumes ranged from 0.5 to 15 mL). Table 3 lists the equilibrium constants determined and critical concentrations (including ionic strength and buffer capacity) calculated in the various assays.

The pK_a values of the model compounds are listed in Table 1 at standard state conditions ($I_{ref} = 0.15$ M, 25 °C), but the primed constants in Table 3 are those that were actually applied at the specific ionic strength (0.25 – 4.34 M), calculated as described in Appendix B. Those of diprenorphine changed slightly by 0.0 to +0.05 (pK_{a1}) and 0.0 to -0.04 (pK_{a2}); that of codeine +0.02 to +0.14; that of lidocaine +0.15 to +0.51. According to the simple Debye-Hückel theory, those of monoprotic bases are expected not to change, but Eq. B5 predicts otherwise. Also listed in Table 3 are the actual pK_{sp} values used at the particular ionic strengths, compared to those at I_{ref} .

Table 1. Physicochemical properties, 25 °C.

Compound	MW	log P	pK _a	Literature -log S ₀ = pS ₀ [M]
Diprenorphine	425.56	3.81 ^a	9.68 ± 0.01 ^d , 8.52 ± 0.01 ^d	--
Codeine	299.36	1.19 ^b	8.24 ^b	1.52 ^e
Lidocaine	234.34	2.44 ^c	7.95 ^c	2.04 ^f , 1.88 ^g , 1.75 ^h , 1.71 ⁱ

^a Calculated using ADME Boxes 4.9 (ACD Labs, Toronto). ^b[22] ^c [23] ^d This work.

^e[24] ^f[16]. ^g[25] ^h[26] ⁱ[27]

The solubility products are also affected significantly when $I \gg I_{ref}$. For example, the phosphate pK_{sp} of codeine phosphate decreased from 1.00 {1.11} at I_{ref} to -0.07 {0.02} at $I = 1.21$ {1.07} M, where the braced value refers to Britton-Robinson universal and the unbraced value refers to Sørensen buffer measurements (Table 3). Lidocaine showed similar large shifts due to the large $I = 4.34$ {3.81} M. The changes were much smaller for the codeine chloride pK_{sp}, since $I = 0.33$ {0.24} M.

Table 2. Shake-flask solubility data, 25 °C^a

pH	Buffer	V (14.85 M H ₃ PO ₄) /ml ^b	V (0.5 M KOH) /ml ^b	S (mg mL ⁻¹)	Sample Wt. (g mL ⁻¹)	Expected Precipitate
0.83	Sör	0.4998	0.0000	4.98 ± 0.04	0.0307	XH ₂ ⁺ H ₂ PO ₄ ⁻ (s)
1.94	Sör	0.0111	0.0000	54.1 ± 1.1	0.0601	XH ₂ ⁺ Cl ⁻ (s)
3.53	Sör	0.0003	0.0000	45.2 ± 0.9	0.0634	XH ₂ ⁺ Cl ⁻ (s)
5.06	Sör	0.0000	0.0082	45.1 ± 1.2	0.0637	XH ₂ ⁺ Cl ⁻ (s) + XH(s)
7.22	Sör	0.0000	0.4969	0.249 ± 0.014	0.0050	XH(s)
8.34	Sör	0.0000	0.5702	0.088 ± 0.021	0.0114	XH(s)
8.83	Sör	0.0000	0.5759	0.032 ± 0.001	0.0007	XH(s)
10.38	Sör	0.0000	0.6251	0.667 ± 0.008	0.0106	XH(s)
11.52	Sör	0.0000	0.8274	4.79 ± 0.11	0.0051	XH(s)
5.00	BR	0.0000	0.1376	52.0 ± 0.1	0.1050	BH ⁺ Cl ⁻ (s)
5.00	Sör	0.0000	0.0044	53.0 ± 0.1	0.1535	BH ⁺ Cl ⁻ (s)
5.00	BR	0.0000	0.1716	290 ± 38	0.4950	BH ⁺ H ₂ PO ₄ ⁻ (s)
5.00	Sör	0.0000	0.0390	308 ± 30	0.5305	BH ⁺ H ₂ PO ₄ ⁻ (s)
5.00	BR	0.2149	0.0000	310 ± 15	0.7700	BH ⁺ H ₂ PO ₄ ⁻ (s)
5.00	Sör	0.2940	0.0000	294 ± 10	1.0300	BH ⁺ H ₂ PO ₄ ⁻ (s)
5.00	BR	0.0000	0.0064	>1000	1.0300	BH ⁺ Cl ⁻ (s)
5.00	Sör	0.0000	0.0042	>1000	1.1354	BH ⁺ Cl ⁻ (s)

^a Sör = Sørensen buffer (0.15 M phosphate, adjusted with NaOH). BR = Britton-Robinson universal buffer (acetic, phosphoric, and boric acids, each at 0.04 M, adjusted with NaOH to pH 5). ^b Calculated by pDISOL-X.

The pK_a values of the acetate, phosphate, and borate buffer components used were taken from Wiki-pK_a website (<http://www.in-ADME.com/wiki_pka.php/>). These were automatically adjusted for changes in the ionic strength by pDISOL-X. The changes were most pronounced with phosphoric acid (0.17 – 0.62), and were minimal with acetic and boric acids (0.01-0.06).

Table 3 - Equilibrium Constants (24 h incubation, 25°C)^a

pH		Buffer Capacity (mM/pH)	Ionic Str. (M)	pK _a ' (base)	pK _a ' (acid)	pK _{sp} ' (phosphate)	pK _{sp} ' (chloride)	[BH ⁺] or [XH ₂ ⁺]	[Cl ⁻]	[H ₂ PO ₄ ⁻]	[BH][Cl] or [XH ₂][Cl]	[BH][H ₂ PO ₄] or [XH ₂][H ₂ PO ₄]	pK _{sp}	pS ₀
Diprenorphine Hydrochloride														
0.83	Sör	1183	0.40	8.55	9.65		1.96	0.088	0.088	0.314	0.008	0.028	2.06 ± 0.07	4.60 ± 0.08
1.94	Sör	242	0.26	8.54	9.66		2.01	0.099	0.099	0.163	0.010	0.016		
3.53	Sör	12	0.25	8.53	9.67		2.02	0.098	0.098	0.150	0.010	0.015		
5.06	Sör	8	0.25	8.53	9.66		2.01	0.098	0.099	0.146	0.010	0.014		
7.22	Sör	169	0.34	8.55	9.65		1.98	0.001	0.093	0.026	0.000	0.000		
8.34	Sör	122	0.37	8.52	9.68		1.97	0.000	0.088	0.002	0.000	0.000		
8.83	Sör	118	0.37	8.55	9.65		1.97	0.000	0.088	0.001	0.000	0.000		
10.38	Sör	87	0.40	8.55	9.65		1.96	0.000	0.086	0.000	0.000	0.000		
11.52	Sör	46	0.51	8.57	9.64		1.93	0.000	0.076	0.000	0.000	0.000		
Codeine Hydrochloride														
5.00	BR	20	0.24	8.26		b	1.52	0.173	0.173	0.037	0.030	0.006	1.57	1.52
5.00	Sör	6	0.33	8.27		b	1.51	0.177	0.177	0.147	0.031	0.026	1.59	1.52
Codeine Phosphate														
5.00	BR	69	1.07	8.38		0.02	c	0.967	0.000	0.984	0.000	0.952	1.11	1.52
5.00	Sör	52	1.21	8.38		-0.07	c	1.026	0.000	1.152	0.000	1.182	1.00	1.52
Lidocaine (free base)														
5.00	BR	102	1.34	8.13		-0.22	c	1.319	0.000	1.248	0.000	1.647	0.88	2.04
5.00	Sör	87	1.39	8.10		-0.22	c	1.250	0.000	1.318	0.000	1.647	0.85	2.04
Lidocaine Hydrochloride														
5.00	BR	63	3.81	8.43		b	-1.16	3.789	3.789	0.021	14.357	0.078	-0.85 ^d	2.04
5.00	Sör	6	4.34	8.46		b	-1.24	4.184	4.184	0.148	17.504	0.618	-0.99 ^d	2.04

^aConcentrations are in molar units. Sör = Sörensen buffer. BR = Britton-Robinson universal buffer, X = ampholyte anion, B = free base. Primed constants refer to the actual ionic strength and are those used in the calculations. Non-primed constants are normalized to values at $I_w = 0.15$ M. ^bSolubility product not exceeded. ^cChloride salt not possible since assay is chloride-free. ^dBased on minimum possible lidocaine chloride solubility.

Diprenorphine Hydrochloride

Figure 1a shows the logS vs. pH profile of diprenorphine hydrochloride (XH₂⁺Cl⁻). The dashed line is calculated with the Henderson-Hasselbalch equation (Eq. A5), using the two pK_a of diprenorphine. The solid curve was calculated based on the equilibrium model most consistent with the actual logS measurements at the various pH. For pH > pK_a^{GIBBS} (5.07; cf., Appendix A), the precipitate is the uncharged ordinary ampholyte, showing the characteristic parabolic shape. The intrinsic solubility (molar units) was refined as pS₀ = 4.60 ± 0.08 (S₀ = 11.5 ± 0.4 μg mL⁻¹).

At pH below the Gibbs pK_a, either the chloride or the phosphate salt precipitates (or possibly both). It is not easy to be certain which form precipitates unless several measurements are made at pH < 2, in order to exploit the common ion effect which would be evident for phosphate salt, since phosphoric acid was used to lower the pH. The solubility at pH 0.83 suggests that a phosphate precipitate may form at the very low pH. In contrast, the chloride model predicts that all solid dissolves at pH 0.83 (given the amount of sample added). The flat shape of the curve pH 2–5 is most consistent with chloride precipitate. A phosphate precipitate would be expected to show an upward curvature near the Gibbs pK_a. The constant based on the assumed chloride precipitate is pK_{sp} = 2.06 ± 0.07 (S_{XH} = 44.6 ± 1.5 mg mL⁻¹, in hydrochloride equivalents).

At pH > 9, the logS – pH curve shows a shift to lower pH, compared that what would be predicted from the Henderson-Hasselbalch equation. The consistent interpretation of the shift is that a water-soluble mixed-charge anionic dimer forms, with the stoichiometry XHX⁻ [19]. Similar species have been observed in numerous studies, and in some cases, LC/MS was able to corroborate the hypothesized aggregate formations [18]. The equilibrium constant for the reaction XH + X⁻ = XHX⁻ is log K_{XHX} = 5.21 ± 0.18 M⁻¹.

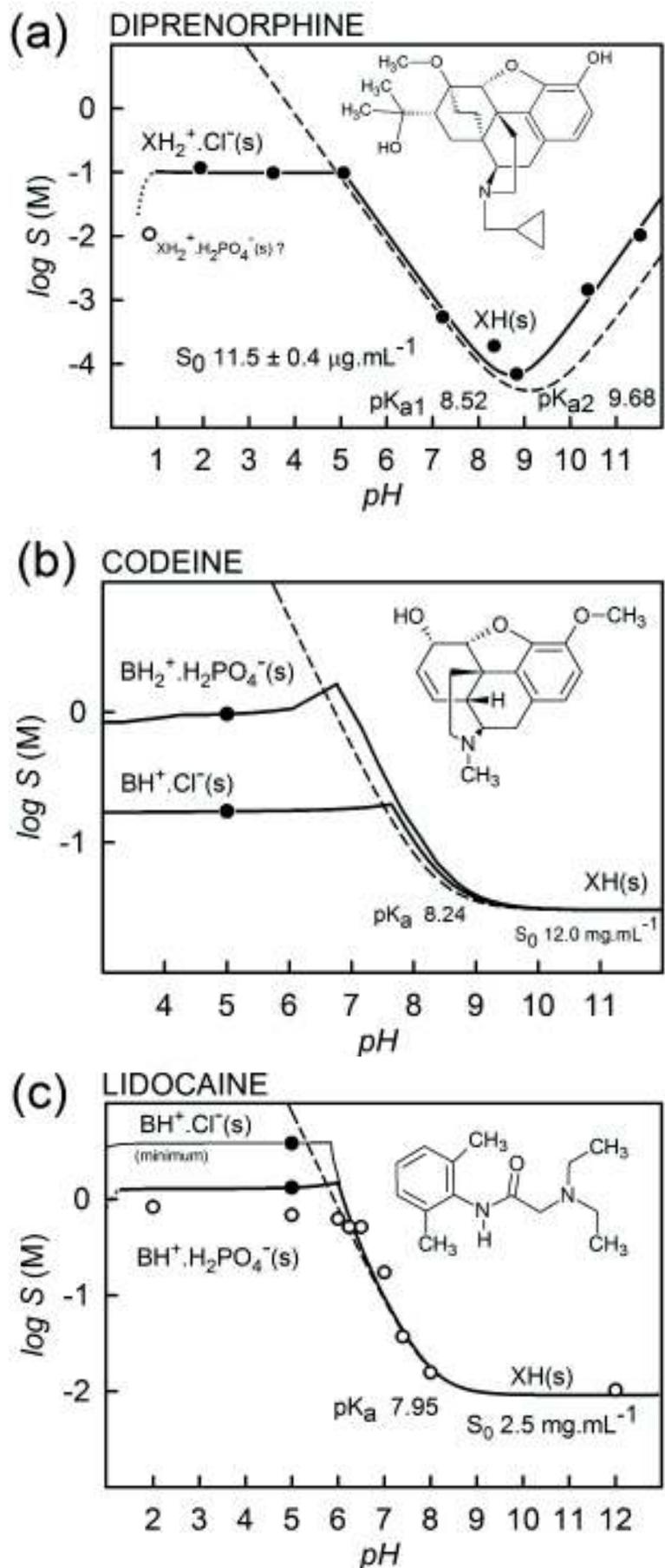


Figure 1. Solubility-pH profiles of the three model drugs studied. (See text)

Codeine Hydrochloride and Dihydrogenphosphate

Figure 1b shows the codeine hydrochloride (BH^+Cl^-) and phosphate ($\text{BH}^+\text{H}_2\text{PO}_4^-$) $\log S - \text{pH}$ profiles. The curve was calculated assuming the intrinsic solubility, $\text{p}S_0 = 1.52$ ($S_0 = 12 \text{ mg mL}^{-1}$, assuming phosphate salt molecular weight), reported by Kuhne *et al.* [24]. Although single pH measurements were made, the whole curve adds useful perspective to the expected solubility – pH relationship. The assignment of the types of salts formed is possible in the way the assays were designed. The codeine phosphate assay had no source of chloride, and thus revealed the phosphate $\text{p}K_{\text{sp}} = 1.00$ {1.11}. The codeine chloride assay indicated a significantly higher $\text{p}K_{\text{sp}} = 1.59$ {1.57}, suggesting that the salt precipitate was that of the chloride. Furthermore, the solubility product for the phosphate salt in the case of the chloride was not exceeded (Table 3), suggesting the absence of phosphate salt in the chloride assay.

Lidocaine Free Base and Hydrochloride

Figure 1c shows the lidocaine phosphate ($\text{BH}^+\text{H}_2\text{PO}_4^-$) $\log S - \text{pH}$ profile. The chloride was very soluble with $S_{\text{BH}} > 1 \text{ g mL}^{-1}$. The chloride $\text{p}K_{\text{sp}}$ in Table 3 refer to the minimum possible values: $\text{p}K_{\text{sp}}^{\text{BHCl}} = -0.99$ {-0.85}, suggesting that the chloride salt is at least 50 times more soluble than the phosphate salt. The solid curve was calculated assuming the intrinsic solubility, $\text{p}S_0 = 2.04$ ($S_0 = 2.5 \text{ mg mL}^{-1}$, assuming BH^+Cl^- molecular weight), reported by Bergström *et al.* [16]. As in the case of the codeine assays, the assignment of the types of salts formed with lidocaine is possible in the way the assays were designed. The lidocaine free base assay had no source of chloride, and thus revealed the phosphate $\text{p}K_{\text{sp}} = 0.85$ {0.88}. The solubility product for the phosphate salt in the case of the chloride was not exceeded (Table 3), suggesting the absence of phosphate salt in the chloride assay.

The unfilled circle symbols in Figure 1c are the solubility values reported by Bergström *et al.* [16], using the miniaturized shake-flask method (25 °C, 0.15 M phosphate buffer, 24 h incubation).

Even though the $\text{p}K_{\text{sp}}$ values of phosphate precipitate in the free base case were nearly identical for the two buffer systems used, the morphology of the crystals isolated was quite different, as shown in Figure 2, with the Sørensen buffer producing much larger and better-formed crystals, compared to the Britton-Robinson buffer (approx. 800 vs. 60 μm , respectively). The X-ray powder diffractograms in Figure 3 confirmed that the crystals formed in the free base assay were those of the same phosphate salt polymorph.

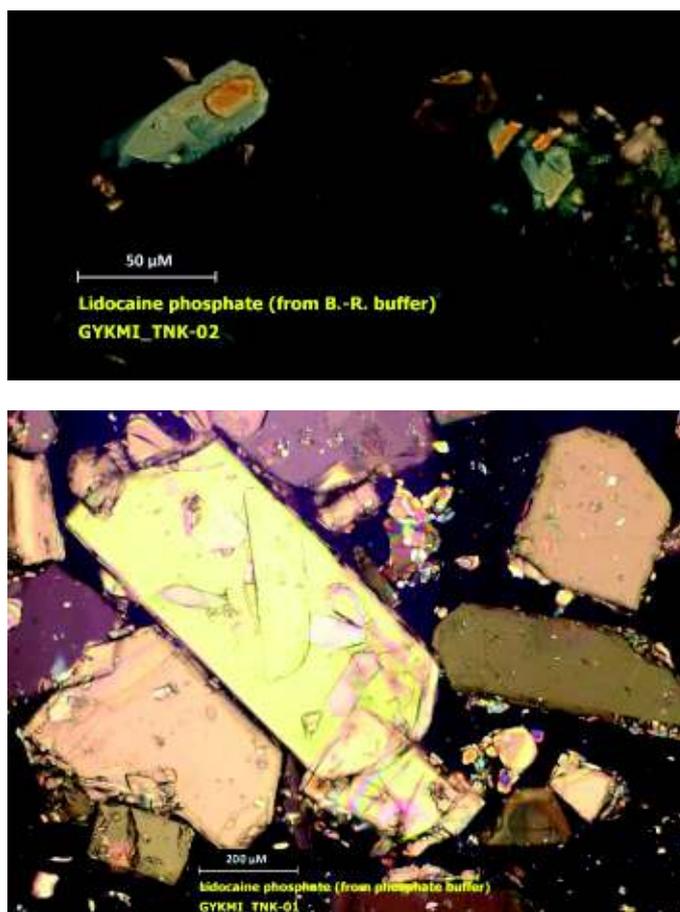


Figure 2. Microphotographs of lidocaine phosphate solids obtained from solubility experiments: (a) Britton-Robinson buffer, (b) Sörensen buffer.

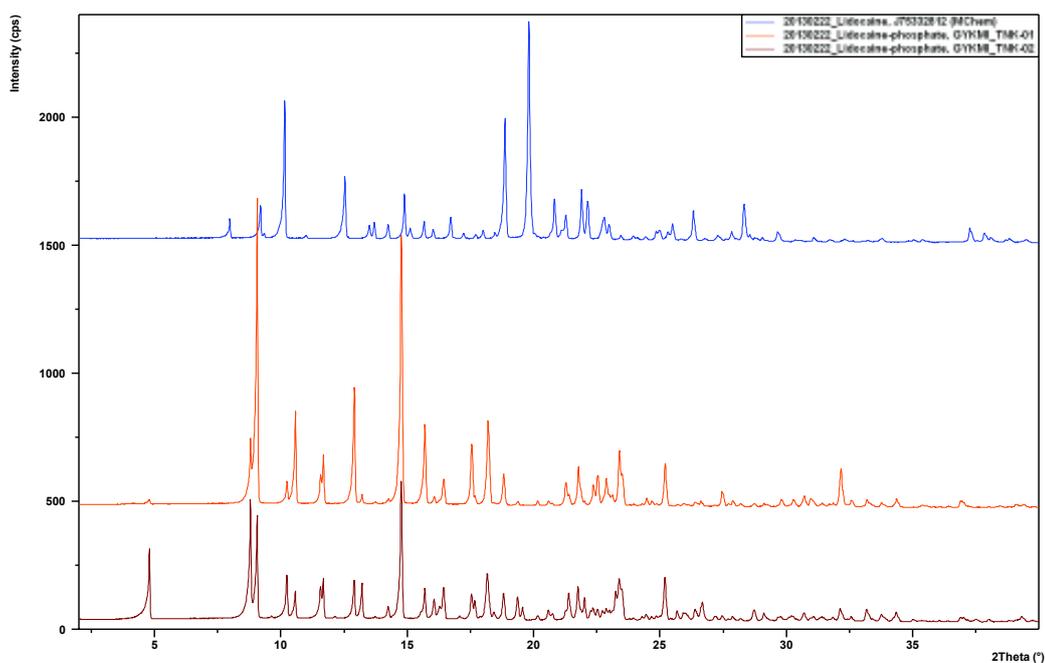


Figure 3. X-ray powder diffractograms of reference lidocaine base (blue), lidocaine phosphate from solubility measurement in Britton-Robinson buffer (brown) and in Sörensen buffer (orange).

Conclusion

The design of salt solubility assays here and the data analysis capability of the new program, *pDISOL-X* offered an opportunity to critically investigate issues related to the challenges of characterizing salt solubility products, such as the high ionic strengths, which can affect values of equilibrium constants and the calibration of pH electrodes. The “anomalies” in the shapes of $\log S - \text{pH}$ profiles that cannot accurately be predicted by the Henderson-Hasselbalch may be common with sparingly-soluble or practically-insoluble drugs, such as diprenorphine, but are not always easy to recognize unless accurately-determined $\text{p}K_a$ values are available [28]. Phosphate buffers can dramatically influence the solubility profiles of ionizable drugs, as shown here and elsewhere [16,29]. These and other similar complications may be common, but are not always easy to interpret quantitatively. In such instances, *pDISOL-X* may be a helpful data analysis and simulation tool. It can further aid in the analysis of dissolution mechanisms which depend on the salt solubility of drugs.

Appendices

A) Explicit Solubility - pH Equations

The idealized relationship between solubility and pH can be readily derived for simple equilibrium models. The "model" refers to a set of equilibrium equations and the associated equilibrium constants. The following are examples of such derivations correspond to a diprotic ampholyte.

Precipitation of Diprotic Amphoteric Drug, XH (Henderson-Hasselbalch Equation)

In the case of a diprotic amphoteric drug, a saturated solution can be defined by the equations and the corresponding constants



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:

$$S = [\text{X}^-] + [\text{XH}] + [\text{XH}_2^+] \quad (\text{A4})$$

where the square brackets denote molar concentration of species. The above equation can be transformed into an expression containing only constants and $[\text{H}^+]$ (as the only variable), by substituting the ionization and solubility Eqs. A1-A3 into Eq. A4.

$$\begin{aligned} \log S &= \log(K_{a1}/([\text{XH}][\text{H}^+]) [\text{XH}] [\text{H}^+] / K_{a1} + [\text{XH}] + [\text{XH}][\text{H}^+] / K_{a1}) \\ &= \log[\text{XH}] + \log(K_{a2} / [\text{H}^+] + 1 + [\text{H}^+] / K_{a1}) \\ &= \log S_0 + \log(1 + 10^{-\text{p}K_{a2} + \text{pH}} + 10^{+\text{p}K_{a1} - \text{pH}}) \end{aligned} \quad (\text{A5})$$

Eq. A5 is often called the Henderson-Hasselbalch (HH) equation for a diprotic ampholyte, and describes a U-shaped $\log S - \text{pH}$ curve. At the low-pH bend in the $\log S - \text{pH}$ curve, the pH equals the $\text{p}K_{a1}$; at the other bend, pH equals the $\text{p}K_{a2}$.

Salt Precipitation of Diprotic Amphoteric Drug, XH

Eq. A5 considers only one precipitate, that of the uncharged species XH, defining the “intrinsic solubility” concentration, S_0 . If the solubility experiment were carried out as an acid-base (HCl/NaOH) titration, over a wide range $\text{pH} \ll \text{p}K_{a1}$ to $\text{pH} \gg \text{p}K_{a2}$, with enough excess compound added that the solubility products of the salts $\text{XH}_2^+\text{Cl}^-(\text{s})$ and $\text{Na}^+\text{X}^-(\text{s})$ are exceeded, then the curve would have regions at low pH and high pH that would not behave as indicated by the HH equation. To describe these processes, two additional solubility equations need to be added to Eqs. A1-A3:



Hydrochloride Salt of the Drug, $\text{XH}_2^+\text{Cl}^-(\text{s})$

If enough compound is added to the suspension and the pH is gradually lowered, well below $\text{p}K_{a1}$, at a critical pH point, called $\text{p}K_{a1}^{\text{Gibbs}}$ [30,31], the HH equation is replaced by an approximately horizontal line.

In the salt solubility region, $\text{pH} < \text{p}K_{a1}^{\text{Gibbs}}$, Eq. A4 still holds, but $[\text{XH}_2^+]$ becomes constant, instead of $[\text{XH}]$. Eq. A5 can be modified (using Eqs. A1, A2 and A6) to reflect this.

$$\begin{aligned} \log S &= \log(K_{a2}K_{a1}[\text{XH}_2^+] / [\text{H}^+]^2 + K_{a1}[\text{XH}_2^+] / [\text{H}^+] + [\text{XH}_2^+]) \\ &= \log[\text{XH}_2^+] + \log(1 + 10^{-\text{p}K_{a1}-\text{p}K_{a2}+2\text{pH}} + 10^{-\text{p}K_{a1}+\text{pH}}) \\ &= \log K_{\text{sp1}} - \log[\text{Cl}^-] + \log(1 + 10^{-\text{p}K_{a1}-\text{p}K_{a2}+2\text{pH}} + 10^{-\text{p}K_{a1}+\text{pH}}) \end{aligned} \quad (\text{A8})$$

For $\text{pH} \ll \text{p}K_{a1}$, the right-most logarithmic term in Eq. A8 becomes vanishingly small, and cationic-drug *conditional* solubility is described by $S_{(+)} = [\text{XH}_2^+] = K_{\text{sp1}} / [\text{Cl}^-]$. Eq. A8 largely describes a horizontal line. However, since the HCl titrant both dilutes the solution and elevates the Cl^- , the horizontal line curves downward for $\text{pH} < 2$, noted as the chloride “common-ion” effect, according to Eq. A8.

Sodium Salt of the Drug, $\text{Na}^+\text{X}^-(\text{s})$

In the presence of a large excess of compound in alkaline $\text{pH} \gg \text{p}K_{a2}$, at the second critical pH point, $\text{p}K_{a2}^{\text{Gibbs}}$, the HH equation is replaced by another approximately horizontal line.

In the salt solubility region, $\text{pH} > \text{p}K_{a2}^{\text{Gibbs}}$, Eq. A4 still holds, but $[\text{X}^-]$ becomes constant, while $[\text{XH}]$ and $[\text{XH}_2^+]$ are variable. Eq. A5 can be further modified (using Eqs. A1, A2 and A7) as

$$\begin{aligned} \log S &= \log([\text{X}^-] + [\text{X}^-][\text{H}^+] / K_{a2} + [\text{X}^-][\text{H}^+]^2 / (K_{a1}K_{a2})) \\ &= \log[\text{X}^-] + \log(1 + 10^{+\text{p}K_{a1}+\text{p}K_{a2}-2\text{pH}} + 10^{-\text{p}K_{a2}+\text{pH}}) \\ &= \log K_{\text{sp2}} - \log[\text{Na}^+] + \log(1 + 10^{+\text{p}K_{a1}+\text{p}K_{a2}-2\text{pH}} + 10^{-\text{p}K_{a2}+\text{pH}}) \end{aligned} \quad (\text{A9})$$

For $\text{pH} \gg \text{p}K_{a2}$, the right-most logarithmic term in Eq. A9 becomes vanishingly small, and *conditional* anionic-drug solubility is described by $S_{(-)} = [\text{X}^-] = K_{\text{sp2}} / [\text{Na}^+]$. Since the NaOH titrant both dilutes the solution and raises the sodium concentration in solution, the horizontal line curves downward for $\text{pH} > 12$, as a result of the sodium “common-ion” effect, according to Eq. A9.

The Complete Solubility Equation

Across the whole pH range, given enough compound excess, the complete solubility equation consists of three segments: (a) *conditional* hydrochloride salt region, $\text{pH} < \text{p}K_{a1}^{\text{Gibbs}}$ (Eq. A8), (b) drug concentration-independent “HH” region, $\text{p}K_{a2}^{\text{Gibbs}} > \text{pH} > \text{p}K_{a1}^{\text{Gibbs}}$ (Eq. A5), and (c) *conditional* sodium salt region, $\text{pH} > \text{p}K_{a2}^{\text{Gibbs}}$ (Eq. (A9)).

Gibbs pK_a Values

At the lower pH = pK_{a1}^{Gibbs}, two solids co-precipitate: XH₂⁺Cl⁻(s) and XH(s). At the higher pH = pK_{a2}^{Gibbs}, two different solids co-precipitate: Na⁺X⁻(s) and XH(s). More discussion of the topic may be found in the literature [19,30,31].

Cationic, Anionic and Neutral Self-Aggregate Formations

In concentrated drug solutions or in solutions containing practically insoluble drugs, oligomeric species, X_nⁿ⁻, (XH₂⁺)_nⁿ⁺, (XH)_n, (XH.X⁻)_nⁿ⁻, (XH.XH₂⁺)_nⁿ⁺, ..., can form. These can characteristically alter the shape of the solubility – pH profile. Consequently, most of Eqs. A1-A9 would need to be modified to accommodate aggregation equilibria. The resultant solubility equations often become very complex, and not all combinations of possible species have had the corresponding equations derived. Examples of such derivations of aggregation-solubility equations may be found in the literature [19].

B) Automatic Ionic Strength Compensation

Generally, the ionic strength, *I*, changes in the course of an acid-base titration due to ionizations, additions of titrant, and dilution of concentrations. This change affects acid-base equilibrium constants. In solubility experiments designed to determine salt solubility products, *K_{sp}*, the ionic strength can vary substantially during a titration, and sometimes reaches values as high as 1 M, or even higher. In contrast, ionization constants, pK_a, are determined at a nearly constant *I* = 0.15 M, under conditions where low sample concentrations (e.g., 10⁻³ to 10⁻⁶ M) are “swamped” by the added inert salt (e.g., 0.15 M NaCl or KCl). The independently-determined pK_a constants are critical to the analysis of solubility data, and thus the above large differences in ionic strength need to be factored in, as described here.

It is a reasonable practice to designate 0.15 M as the “reference” ionic strength, *I_{ref}*, (“physiological” level) to which *all* equilibrium constants are scaled in the solubility assay. In the older literature, the reference state was often chosen as zero, but in current pharmaceutical applications, 0.15 M is usually chosen, with no loss of thermodynamic rigor [19].

Since the ionic strength at any given pH point in a titration is likely different from *I_{ref}*, all ionization constants need to be locally transformed (from reference *I_{ref}* to local *I*) for the calculation of local point concentrations. The procedure below describes such an adjustment of activity coefficients.

Consider a three-reactant system, based on reactants X, Y and H (proton), whose charges are Q_x, Q_y, and +1. The concentration of the jth particular species, C_j, is defined in terms of these reactants

$$C_j = [X^{Q_x}]^{e_{xj}} [Y^{Q_y}]^{e_{yj}} [H^+]^{e_{hj}} \beta_j \quad (B1)$$

associated with the general equilibrium expression



where β_j is the cumulative formation constant [19], and e_{xj}, e_{yj}, and e_{hj} are the X, Y, and H stoichiometric coefficients, respectively, of the jth species.

At each pH point in a solubility assay, *I* is calculated precisely, according to the general formula [32]:

$$I = [\text{NaCl}] + [\text{NaOH}] + [H^+] + \frac{1}{2} \{ Q_x(1+Q_x) x + Q_y(1+Q_y) y + \sum Q_j(1+Q_j) C_j \} \\ + \frac{1}{2} \{ |Q_x + n_x| - Q_x - n_x \} X + \frac{1}{2} \{ |Q_y + n_y| - Q_y - n_y \} Y \quad (B3)$$

The last two terms in braces in Eq. B3 take into account any n_x or n_y number of counter ions introduced to the solution by drug substances in salt form (per X- or Y-compound, respectively).

The reference set of $\beta(I_{ref})$ formation constants are locally transformed to the set $\beta(I)$ according to the general expression:

$$\log \beta_j(I) = \log \beta_j(I_{ref}) + e_{xj} \log \left[\frac{f_x(I)}{f_x(I_{ref})} \right] + e_{yj} \log \left[\frac{f_y(I)}{f_y(I_{ref})} \right] + e_{hj} \log \left[\frac{f_h(I)}{f_h(I_{ref})} \right] - \log \left[\frac{f_j(I)}{f_j(I_{ref})} \right] \quad (B4)$$

where $\beta(I)$ refers to the ionic strength I , while $\beta(I_{ref})$ refers to I_{ref} . The ionic-strength-dependent activity coefficients of X, Y, H, and j^{th} species (cf., Eq. B2) are denoted f_x , f_y , f_h , and f_j , respectively. A similar equation was introduced by Avdeef [32], based on the Davies-modified Debye-Hückel equation, which is reasonably useful up to $I = 0.3$ M. Since much higher ionic strengths are reached in salt solubility experiments, Eq. B4 in the current study is cast in an expanded activity coefficient equation based on the hydration theory proposed by Stokes and Robinson [32,33], further elaborated by Bates *et al.* [34] and Robinson and Bates [35] to include single-ion activities, then slightly modified by Bockris and Reddy [36], and recently applied to solubility data of a drug-like molecule by Wang *et al.* [7]:

$$\log f_i(I) = -Q_i^2 \frac{A\sqrt{I}}{1 + B\hat{a}_i\sqrt{I}} - h_i \log a_{H_2O} + \log \left(\frac{C_{H_2O} + \sum_j C_j}{C_{H_2O} + \sum_j (1 - h_j) \cdot C_j} \right) \quad (B5)$$

The first term on the right side of Eq. B5 is the Debye-Hückel equation accounting for the ion-ion electrostatic interactions; the second term is related to the decrease in the activity of water due to the work done in immobilizing some of the bulk water to hydrate ions; the third term is related to free energy change of the ions, as their concentrations effectively increase when the volume of bulk water decreases upon hydration of the ions. The parameters at 25°C (molar scale): dielectric constant of water, $\epsilon = 78.3, 76.8, 67.3$ at $I = 0, 0.15, \text{ and } 1$ M (NaCl), respectively [37]; the Debye-Hückel slope, $A = 1.825 \times 10^6 (\epsilon T)^{-3/2} = 0.512, 0.528, 0.642$ at $I = 0, 0.15, \text{ and } 1$ M, resp.; $B = 50.29 (\epsilon T)^{-1/2} = 0.329, 0.333, 0.355$ at $I = 0, 0.15, 1$ M, resp.; T is the absolute temperature (K). The two adjustable parameters are \hat{a}_i , corresponding to the mean diameter of the i^{th} hydrated ion [38], and h_i , the hydration number of the i^{th} ion [37]. The activity of water, $a_{H_2O} = 1.000, 0.995, 0.967$ at $I = 0, 0.15, 1$ M, respectively [36]. $C_{H_2O} = 55.51$ M (concentration of water in pure solvent form, $I = 0$). The summation symbols in Eq. B5 are over all charged species (including the reactants) in the system.

Acknowledgements

We thank Sándor Hosztafi (Semmelweis University, Dept. of Pharmaceutical Chemistry) for the synthesis of diprenorphine hydrochloride sample. We also thank Dénes Janke for the XRPD measurements.

References

- [1] S. Miyazaki, H. Inouie, T. Nadai, T. Arita, M. Nakano, *Chem. Pharm. Bull.* 27 (1979) 1441-1447.
- [2] S. Miyazaki, M. Oshiba, T. Nadai, *J. Pharm. Sci.* 70 (1981) 594-596.
- [3] B.D. Anderson, R.A. Conradi, *J. Pharm. Sci.* 74 (1985) 815-820.
- [4] B.D. Anderson, K.P. Flora, Preparation of water-soluble compounds through salt formation. In: C.G. Wermuth (Ed.). *The Practice of Medicinal Chemistry*. Academic Press, London, 1996, pp. 739-754.
- [5] M.T. Ledwidge, O.I. Corrigan, *Int. J. Pharm.* 174 (1998) 187-200.

- [6] A.T.M. Serajuddin, M. Pudipeddi, Salt selection strategies, in: Stahl PH, Wermuth CG (Eds.), Handbook of Pharmaceutical Salts: Properties, Selection, and Use, Wiley-VCH, Weinheim, 2002, pp. 135-160.
- [7] Z. Wang, L.S. Burrell, W.J. Lambert, *J. Pharm. Sci.* **91** (2002) 1445-1455.
- [8] P.H. Stahl, Salt selection, in: R. Hilfiker (Ed.), Polymorphism in Pharmaceutical Industry, Wiley-VCH, Weinheim, 2006, pp. 309-322.
- [9] T. Higuchi, M. Gupta, L.W. Busse, *J. Amer. Pharm. Assoc. (Sci. Ed.)* **42** (1953) 157-161.
- [10] T.J. Roseman, S.H. Yalkowsky, *J. Pharm. Sci.* **62** (1973) 1680-1685.
- [11] D.J. Attwood, J. Gibson, *J. Pharm. Pharmacol.* **30** (1978) 176-180.
- [12] A. Fini, G. Fazio, G. Feroci, *Int. J. Pharm.* **126** (1995) 95-102.
- [13] Z. Wang, K.R. Morris, B. Chu, *J. Pharm. Sci.* **84** (1995) 609-613.
- [14] Y. Surakitbanharn, R. McCandless, J.F. Krzyzaniak, R.-M. Dannenfelser, S.H. Yalkowsky, *J. Pharm. Sci.* **84** (1995) 720-723.
- [15] W.H. Streng, D.H.-S. Yu, C. Zhu, *Int. J. Pharm.* **135** (1996) 43-52.
- [16] C.A.S. Bergström, K. Luthman, P. Artursson, *Eur. J. Pharm. Sci.* **22** (2004) 387-398.
- [17] A. Avdeef, *Adv. Drug Deliv. Rev.* **59** (2007) 568-590.
- [18] E. Shoghi, E. Fuguet, E. Bosch, C. Ràfols, *Eur. J. Pharm. Sci.* **48** (2013) 291-300.
- [19] A. Avdeef, *Absorption and Drug Development, Second Edition*. Wiley-Interscience, Hoboken, NJ, 2012.
- [20] A. Avdeef, J.J. Bucher, *Anal. Chem.* **50** (1978) 2137-2142.
- [21] E. Baka, J. Comer, K. Takács-Novák, *J. Pharm. Biomed. Anal.* **46** (2008) 335-341.
- [22] A. Avdeef, D.A. Barrett, P.N. Shaw, R.D. Knaggs, S.S. Davis, *J. Med. Chem.* **39** (1996) 4377-4381.
- [23] A. Avdeef, K.J. Box, J.E.A. Comer, C. Hibbert, K.Y. Tam, *Pharm. Res.* **15** (1997) 208-214.
- [24] R. Kuhne, R-U. Ebert, F. Kleint, G. Schmidt, G. Schuurmann, *Chemosphere* **30** (1995) 2061-2077.
- [25] E. Shoghi, E. Fuguet, C. Ràfols, E. Bosch, *Chem. Biodivers.* **6** (2009) 1789-1795.
- [26] C.M. Wassvik, A.G. Holmén, R. Draheim, P. Artursson. LogP-independent solubility of drugs - molecular descriptors for solid-state limited solubility. In: C.M. Wassvik. PhD Thesis, Univ. Uppsala, 2006.
- [27] S. Pinsuwan, P.B. Myrdal, Y.-C. Lee, S.H. Yalkowsky, *Chemosphere* **35** (1997) 2503-2513.
- [28] G. Völgyi, E. Baka, K. Box, J. Comer, K. Takács-Novák, *Anal. Chim. Acta* **673** (2010) 40-46.
- [29] W.H. Streng, S.K. Hsi, P.E. Helms, H.G.H. Tan, *J. Pharm. Sci.* **73** (1984) 1679-1684.
- [30] A. Avdeef, *Pharm. Pharmacol. Commun.* **4** (1998) 165-178.
- [31] W.H. Streng, *Int. J. Pharm.* **186** (1999) 137-140.
- [32] A. Avdeef, *J. Pharm. Sci.* **82** (1993) 183-190.
- [33] R.H. Stokes, R.A. Robinson, *J. Amer. Chem. Soc.* **70** (1948) 1870-1878.
- [34] R.G. Bates, B.R. Staples, R.A. Robinson, *Anal. Chem.* **42** (1970) 867-871.
- [35] R.A. Robinson, R.G. Bates, *Marine Chem.* **6** (1978) 327-333.
- [36] J.O'M. Bockris, A.K.N. Reddy, *Modern Electrochemistry, Vol. 1*. Plenum Publishing Corp., New York, NY, 1973.
- [37] R.A. Robinson, R.H. Stokes, *Electrolytic Solutions. 2nd Revised Ed.* Dover Publications, Inc., Mineola, NY, 2002.
- [38] J. Kielland, *J. Amer. Chem. Soc.* **59** (1937) 1675-1678.