pH-Metric Solubility. 2: Correlation Between the Acid-Base Titration and the Saturation Shake-Flask Solubility-pH Methods

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Received October 8, 1999; accepted October 18, 1999

Purpose. The objective of this study was to compare the results of a normal saturation shake-flask method to a new potentiometric acid-base titration method for determining the intrinsic solubility and the solubility-pH profiles of ionizable molecules, and to report the solubility constants determined by the latter technique.

Methods. The solubility-pH profiles of twelve generic drugs (atenolol, diclofenac.Na, famotidine, flurbiprofen, furosemide, hydrochlorothiazide, ibuprofen, ketoprofen, labetolol.HCl, naproxen, phenytoin, and propranolol.HCl), with solubilities spanning over six orders of magnitude, were determined both by the new pH-metric method and by a traditional approach (24 hr shaking of saturated solutions, followed by filtration, then HPLC assaying with UV detection).

Results. The 212 separate saturation shake-flask solubility measurements and those derived from 65 potentiometric titrations agreed well. The analysis produced the correlation equation:

$$\log(1/S)_{\text{titration}} = -0.063(\pm 0.032) + 1.025(\pm 0.011)\ \log(1/S)_{\text{shake-flask}},$$

$$s = 0.20,\ r^2 = 0.978.$$

The potentiometrically-derived intrinsic solubilities of the drugs were: atenolol 13.5 mg/mL, diclofenac.Na 0.82 μg/mL, famotidine 1.1 mg/mL, flurbiprofen 10.6 μg/mL, furosemide 5.9 μg/mL, hydrochlorothiazide 0.70 mg/mL, ibuprofen 49 μg/mL, ketoprofen 118 μg/mL, labetolol.HCl 128 μg/mL, naproxen 14 μg/mL, phenytoin 19 μg/mL, and propranolol.HCl 70 μg/mL.

Conclusions. The new potentiometric method was shown to be reliable for determining the solubility-pH profiles of uncharged ionizable drug substances. Its speed compared to conventional equilibrium measurements, its sound theoretical basis, its ability to generate the full solubility-pH profile from a single titration, and its dynamic range (currently estimated to be seven orders of magnitude) make the new pH-metric method an attractive addition to traditional approaches used by preformulation and development scientists. It may be useful even to discovery scientists in critical decision situations (such as calibrating computational prediction methods).

KEY WORDS: solubility; dissolution; solubility-pH profile; potentiometric; oral absorption.

INTRODUCTION

Reliable measurement of the solubility of ionizable molecules offer significant challenges. This is unfortunate, since the need for the measurement is so widespread, extending from discovery through development. Lead compounds originating from high-throughput screening (HTS) have tended towards higher molecular weights and lipophilicity (1). These molecular traits are often associated with low solubility and poor oral absorption. Although HTS focuses primarily on biological activity, the importance of early optimization for physicochemical properties, such as solubility, is increasingly recognized in the pharmaceutical industry. Beyond discovery, at the preformulation stage, among the first physicochemical parameters to be carefully measured is often the solubility. Solubility data as a function of pH are needed for development of parenteral formulations for use in early animal bioavailability and toxicity studies. Later in development, solubility takes on a broader focus: salt selection, rate of drug dissolution, and stability of the dosage form depend in important ways on the solubility of the candidate molecules (2,3).

The traditional saturation shake-flask procedures for measuring the equilibrium solubility of ionizable molecules are manually intensive, time- and sample-consuming, and may be prone to systematic errors not easily recognized, especially when the pH is not carefully measured, when aggregation or complexation reactions take place (unexpectedly altering spectroscopic properties), or when highly insoluble substances are considered (exceeding the sensitivity of the analytical method). Some of discovery's need for solubility information has been addressed by a fast approximate "kinetic" methods of solubility measurements, based on turbidimetric analysis, as described by Lipinski (1). Recently a new potentiometric method has been proposed which overcomes some of the limitations of standard approaches as well (4). Although a few potentiometric determinations of solubility have been reported (5–8), comparative evaluation of this method with reference to traditional methods of solubility determination has not been presented in the literature.

Use of solubility information in the regulatory decision making process has increased over the last few years. FDA-supported research led to the development of the Biopharmaceutics Classification System (BCS) (9). The BCS allows estimation of the likely contributions of three major factors, dissolution, solubility, and intestinal permeability, which affect oral drug absorption from immediate release (IR) solid oral products. The scheme was first introduced into the regulatory decision-making process through the "Scale-Up and Post Approval Change Guidance for Immediate Release Solid Oral Dosage Forms (10)." A recent draft guidance document proposes to further expand the regulatory applications of the BCS and recommends methods for classifying drugs and IR drug products (11).

In the present study, the solubility-pH profiles of twelve generic drugs (acids, bases and ampholytes), spanning six orders of magnitude in solubilities, were determined both by the new pH-metric method and by a traditional shake-flask approach, where concentrations were determined by HPLC with UV detection, following procedures described in the US Pharmacopeia (12). The results of the two independent techniques were compared. Focus was placed on determination of the aqueous solubilities under conditions where only the uncharged molecule precipitates.

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0724-8741/00/0100-0085$18.00/0

MATERIALS AND METHODS

Chemicals

The preparation and standardization of titrants (0.5M HCl and 0.5M KOH) and the special calibration of the pH electrode are described elsewhere (13,14). Atenolol, diclofenac (sodium), famotidine, flurbiprofen, furosemide, hydrochlorothiazide, ibuprofen, ketoprofen, labetolol (hydrochloride), naproxen (acid), phenytoin, and propranolol (hydrochloride) were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and used as received. Standard buffers (HCl/NaCl pH 1–3, phosphate pH 3–10, NaOH/NaCl pH 10–12) for the saturation shake-flask procedure were prepared from US Pharmacopeia methods (12).

Apparatus

The ionization constants (pK_a) and the octanol-water partition coefficients (log P) of the compounds were measured using the PCA101 and the GLpKa instruments (Sirius Analytical Instruments Ltd., Forest Row, E. Sussex, UK).

The potentiometric solubility data were obtained with the *p*SOL Model 3 instrument (*p*ION INC., Cambridge, MA, USA) and subsequently processed with the accompanying computer program, *p*S. The new solubility instrument is equipped with three precision dispensers (capable of adding a minimum volume of 0.02 μL) and a high-impedance (10^{15} Ω) pH circuit. The potentiometric titrations were performed under a blanket of argon gas flow, at 25°C (sample vial in a glass jacket vessel with temperature controlled by a circulating bath, ±0.2°C), in solutions containing 0.15 M KCl. The method can accommodate sample weights as low as 50–100 μg (for low-solubility compounds). Care was taken to ensure that the amount of sample used was less than a thousand times the predicted minimum sample solubility, to avoid chloride or potassium salt precipitation of the drug, due to the presence of 0.15 M KCl. Titrated solution volumes ranged from 1.7 to 17 mL. A teflon-coated magnetic stir bar was used to agitate the titrated solution. When solution volumes are greater than 3 mL, stirring is further assisted by a stream of argon bubbles. A semi-micro combination pH electrode (Ag/AgCl, annular ring single junction) was used in the solubility measurements (*p*ION INC., Cambridge, MA, USA).

The measurement of solubility as a function of pH by the saturation standard methods used an automated HPLC system (Thermo Separation Products, San Jose, CA), tied to a Hewlett Packard 8452A diode array UV/VIS spectrophotometer.

Template Titration Procedure

The *p*SOL instrument takes as input parameters the measured pK_a and the measured octanol-water partition coefficient. The log P parameter is used to estimate the intrinsic solubility, S_o, using the Hansch expression[15]

$$\log (1/S_o) = 1.38 \log P - 1.17 \qquad (1)$$

Using the pK_a and the estimated S_o, the instrument simulates the entire titration curve before starting an assay. The simulated curve serves as a template from which the instrument "learns" how to collect individual pH measurements in the course of the titration. It is not necessary that Eq. (1) be very accurate, since the software in the pSOL instrument can easily tolerate errors of an order of magnitude.

The pH domain embracing precipitation is apparent from the simulation, and data collection strategy is set accordingly. Enough sample is weighed to cause precipitation during some portion of the titration. For compounds with predicted $S_o <$ 500 μg/mL, the solid is first rapidly dissolved in either strong acid or strong base, then quickly re-precipitated and allowed to gestate for about 10–60 min, depending on predicted S_o, before the actual titration starts (during which time crystals often form out of the turbid suspension). Titrations of weak acids begin at low pH and those of weak bases begin at high pH. KOH (or HCl) titrant is dispensed accurately and slowly into the slurry, to drive the pH of the solution in the direction of dissolution, eventually well past the point of complete dissolution. This protocol (starting in the midst of precipitation) very effectively avoids supersaturation of the uncharged species.

As titrant is added, careful measurements of pH are made. The instrument dramatically slows down the rate of data taking as the point of complete dissolution approaches in the titration. The rate of dissolution of the solid is described by the expression (16),

$$\frac{dm}{dt} = A\left(\frac{D}{h}\right)(C_S - C) \qquad (2)$$

where m is the mass (mol), C = concentration of solute dissolved at a particular time (mol/cm^3), C_S = equilibrium solubility (mol/cm^3), D = diffusion coefficient (cm^2/sec), h = apparent thickness (cm) of the diffusion layer (depends on rate of stirring and the temperature), and A = surface area (cm^2) available for dissolution. As the equilibrium state is approached ($C \approx C_S$), the time needed to dissolve additional solid exponentially increases. The *p*SOL instrument directly takes this into account. About 20% of the data, nearest the point of complete dissolution, are allotted about 80% of the time. In this way, problems of incomplete dissolution are either entirely eliminated or at least are easily identified by the shape of the resultant distortion in the actual titration curve. Only after the precipitate completely dissolves (assessment based on the template), does the instrument collect the remainder of the data rapidly, in a manner characteristic of regular titrators. Typically, 3–10 hr are required for the entire equilibrium solubility data taking. The more insoluble the compound is anticipated to be (based on the template) the longer the recommended assay time. In an ideally designed assay (i.e., when the template is accurate to ± 0.5 log unit in solubility), only a single titration is needed to determine the intrinsic solubility constant and the entire solubility-pH profile.

Saturation Shake-Flask Procedure

In the traditional solubility method, the drug is added to a standard buffer solution until saturation occurs, indicated by undissolved excess drug. When necessary, the pH is further adjusted with dilute HCl or NaOH. The flasks are then shaken for a minimum of 24 hr. The amount of dissolved drug is determined by filtering and assaying the supernatant solution by the US Pharmacopeia methods, using HPLC with UV detection (12).

Potentiometric Refinement of Solubility Constants

The graphically determined approximate equilibrium constants (4) produce the "seed" values for the iterative least

Table 1. Potentiometric Titrations

Compound	No. titrations	Concn. range (mM)	Assay time (hr)	Group χ_v
Atenolol	3	50–150	6–12	2.8
Diclofenac.Na	6	0.03–0.4	4–5	2.3
Famotidine	9	5–30	6–10	13.0
Flurbiprofen	9	0.2–1.3	3–5	3.1
Furosemide	2	0.2–0.5	5–8	2.0
Hydrochlorothiazide	3	4–13	5–12	4.2
Ibuprofen	4	0.8–8.5	3–15	2.0
Ketoprofen	5	0.8–3.9	4–7	2.4
Labetolol.HCl	3	4–8	9–17	10.0
Naproxen	9	0.2–1.2	3–6	2.4
Phenytoin	4	0.5–1.4	5–9	5.7
Propranolol.HCl	8	0.2–21	1–13	17.0

squares procedure for log S_o refinement, using the processing program, *p*S. The refined values are those which produce a minimum in the sum of the weighted squares of residuals:

$$S = \sum_{i}^{N_o} \frac{(pH_i^{obs} - pH_i^{calc})^2}{\sigma_i^2\,(pH)} \tag{3}$$

N_o is the number of pH measurements; σ_i^2 is the estimated variance in the measured pH_i^{obs}. The model equation, pH_i^{calc}, is a function of the equilibrium constants, as well as the independent variables. The weighting scheme used in equ. (3) is constructed from the variances (14)

$$\sigma^2\,(pH) = \sigma_c^2 + (\sigma_v\ dpH/dV)^2 \tag{4}$$

where $\sigma_c = 0.005$ (units of pH), the fixed contribution to the variance in the measured pH, and $\sigma_v = 0.00003$ mL, the estimated standard deviation in the volume of titrant. After each iterative cycle a test of the progress of refinement is indicated by the "reduced chi," which is defined by

$$\chi_v = \sqrt{\frac{S}{N_o - N_r}} \tag{5}$$

where N_r is the number of refined parameters. A χ_v value of 1 is ideal for analyses of data from aqueous titrations of unsaturated solutions.

RESULTS AND DISCUSSION

Potentiometric Titrations

Table 1 summarizes some of the characteristics of the titration data. The intrinsic solubility constants were refined (in logarithmic form, based on molarity units) by nonlinear least squares procedure, pooling data from typically five different titrations per compound. In all, 65 titrations were performed. Each titration contained a different sample concentration, chosen according to expected solubilities, and ranged from 30 μM (diclofenac) to 150 mM (atenolol). The more soluble molecules required more sample to effect precipitation. Typically, each titration took 3–10 hr to complete. The reduced-χ (Eq. 5) from grouped refinements ranged from 2 to 17, indicating that, on the average, the experimental titration curves differed from those calculated using the refined equilibrium constants by 0.01 to 0.09 pH units. In this study, we note that on the whole, the potentiometric data are about five times noisier during precipitation, compared to data acquired in the absence of solid formation. This is probably the result of slight interferences with pH readings due to the presence of precipitate and the very long durations of the titrations, with electrode calibration perhaps not being as rigorously constant over the entire interval, compared with conventional titrations.

Table 2. Solubilities[a]

Compound	S_o (intrinsic)	$\log(1/S_o)$ (mol/L) ± std dev	$\log(1/K_{sp})$[b] (mol^2/L^2) ± std dev	logP	pK_a
Atenolol	13.5 mg/mL	1.30 ± 0.12		0.22[c]	9.54[c]
Diclofenac, sodium	0.82 μg/mL	5.59 ± 0.08	2.99 ± 0.01	4.51[d]	3.99[d]
Famotidine	1.1 mg/mL	2.48 ± 0.08		−0.56[e]	11.19, 6.74[e]
Flurbiprofen	10.6 μg/mL	4.36 ± 0.10	2.37 ± 0.05	3.99[f]	4.03[f]
Furosemide	5.9 μg/mL	4.75 ± 0.10		2.56[e]	10.63, 3.52[e]
Hydrochlorothiazide	0.70 mg/mL	2.63 ± 0.05		−0.03[e]	9.96, 8.87[e]
Ibuprofen	49 μg/mL	3.62 ± 0.02	0.10 ± 0.01	4.13[e]	4.42[e]
Ketoprofen	118 μg/mL	3.33 ± 0.05		3.16[e]	3.98[e]
Labetolol.HCl	128 μg/mL	3.45 ± 0.11		1.33[e]	9.42, 7.48[e]
Naproxen	14 μg/mL	4.21 ± 0.01	0.15 ± 0.03	3.24[e]	4.18[e]
Phenytoin	19 μg/mL	4.13 ± 0.11		2.24[e]	8.21[e]
Propranolol.HCl	70 μg/mL	3.62 ± 0.11		3.48[g]	9.53[g]

[a] pH-metric measurements performed at 25°C, in the medium of 0.15 M KCl.
[b] The sodium-salt solubility products reported here, log $1/K_{sp}$, are derived from the saturation shake-flask measurements, which were performed at high enough a concentration to precipitate the salts.
[c] Ref. 17, p. 67.
[d] Ref. 17, p. 146.
[e] Unpublished data.
[f] Ref. 4.
[g] Ref. 17, p. 138.

Refined Intrinsic Solubility Constants, S_o

Table 2 lists the refined solubility constants. The estimated standard deviations in the refined log S_o constants were on the average 0.08, ranging from 0.02–0.12. These are about five times higher than the errors observed in refined pK_a constants, indicating that the data in pH-metric solubility assays are noisier than the data in simple homogenous-media titrations. The constants are also presented in the more popular μg/mL or mg/mL units in Table 2. The intrinsic solubility constants determined here and the associated pK_as determined by normal titrations (Sirius) were used to derive the solubility-pH profiles (4).

Comparisons of Solubility-pH Profiles

Figure 1 shows the solubility-pH profiles of the twelve molecules studied. In the figure, solid curves represent the results of the pH-metric analysis, and points represent 212 individual saturation shake-flask measurements for the twelve molecules. For two of the molecules, flurbiprofen (Fig. 1f) and naproxen (Fig. 1d), we were able to find excellent measurements in the literature (2,8,18,19), which we have also incorporated into the figures. The overall comparison is quite good, as one can see.

Note that the shake-flask measurements for ibuprofen (Fig. 1c), naproxen (Fig. 1d), flurbiprofen (Fig. 1f), and diclofenac (Fig. 1h) for pH > 7 indicate the precipitation of the sodium salts of the weak acids, since very high concentrations of drugs were used in the standard method. The pH-metric method used lower sample concentrations, so salt formations were avoided.

In Figure 2 is a correlation diagram of the pH-metric solubilities plotted against solubilities determined by standard methods. When a theoretical line of zero-intercept, unit-slope is drawn through the points, the resultant standard deviation, $s = 0.20$, which is less than three estimated standard deviations in the determined constants. The linear regression of the data produces a nearly identical s. The calculated intercept $= -0.063 \pm 0.032$, slope $= 1.025 \pm 0.011$, and $r^2 = 0.978$. The correlation plot suggests that the pH-metric method can be a reliable technique for determining the solubilities of ionizable molecules.

CONCLUSIONS

The new automated potentiometric method was shown to be reliable for determining the solubility-pH profiles of

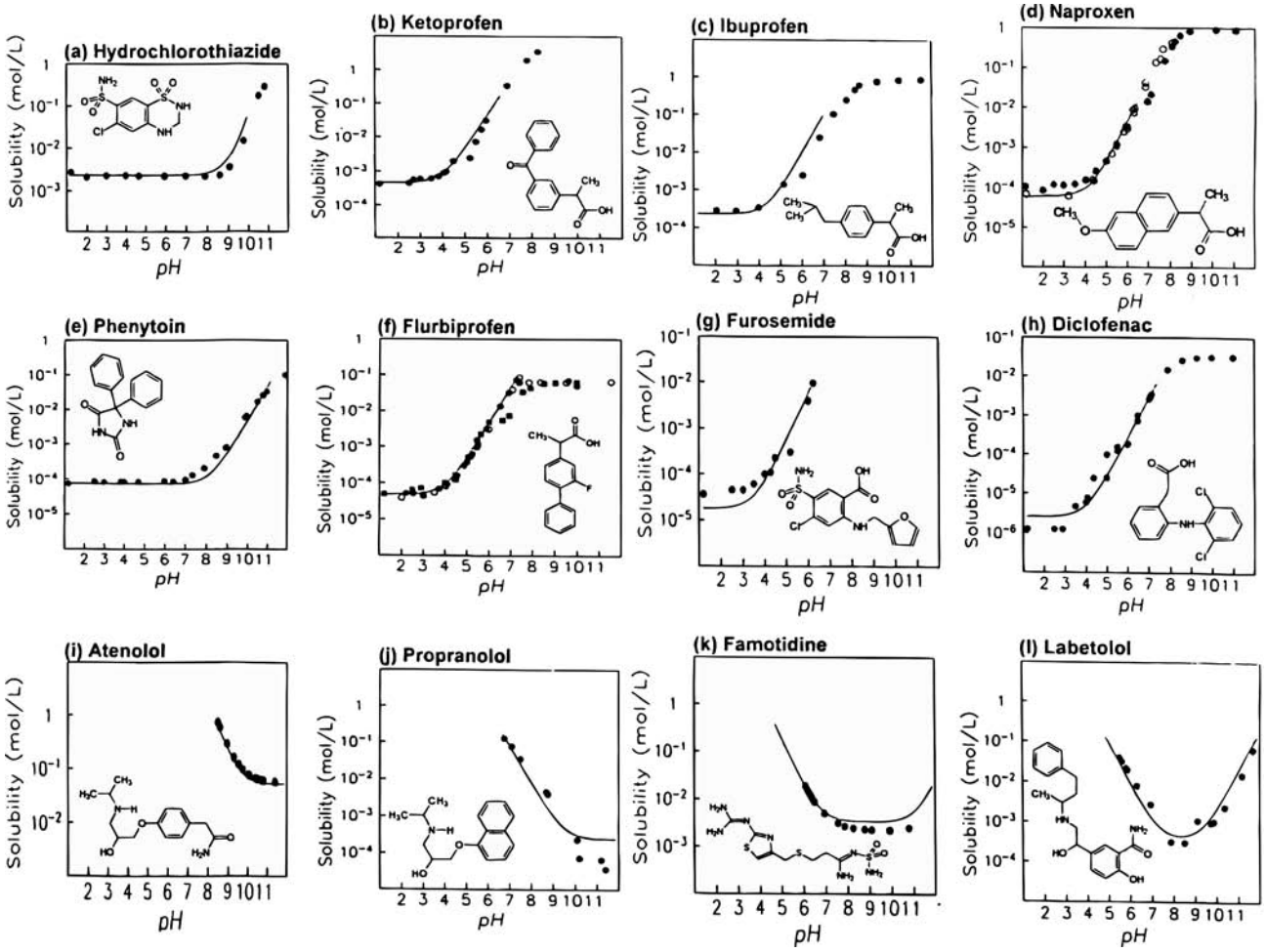

Fig. 1. The solubility-pH profiles of the molecules studied, with solid curves determined by the pH-metric technique and solid circle symbols determined by standard saturation shake-flask methods. The open circles in Fig. 1d (naproxen) represent the data reported in Ref. 19. In Fig. 1f (flurbiprofen), our data are represented by the solid line and the filled squares; open circles are based on data reported in Ref. 18; open squares and filled circles are based on data reported in Ref. 8.

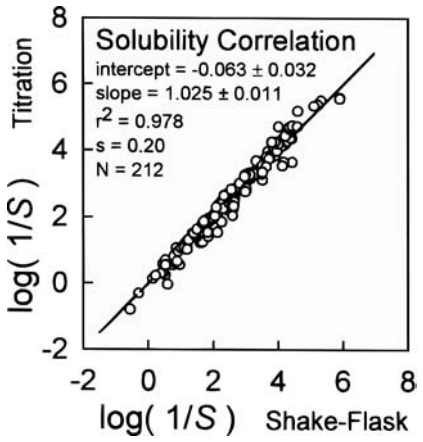

Fig. 2. Correlation graph comparing solubilities determined by the pH-metric technique to the obtained by the standard saturation shake-flask method. The solubilities are shown in logarithmic mol/L units.

uncharged drug substances. Its speed compared to conventional equilibrium measurements (entire solubility-pH profile in 3–10 hr), its sound theoretical basis (4), its ability to generate the full solubility-pH profile from a single titration, and its dynamic range (currently estimated to be seven orders of magnitude) make the new pH-metric method an attractive addition to traditional approaches used by preformulation and development scientists. Although the pH-metric method lacks the high-throughput speed needed in discovery settings, it still may be useful to discovery scientists, for fine-tuning high-volume computational predictions, by pH-metrically characterizing a small number of selected molecules of a congeneric series.

ACKNOWLEDGMENTS

Opinions expressed are those of the authors and may not reflect FDA policies. We are grateful to Drs. Ajaz S. Hussain (FDA), Richard A. Winnike (Glaxo Wellcome), Walt Morozowich and Gordon Halsted (Pharmacia & Upjohn) for illuminating discussions and some assistance. We thank Dr. William M. Meylan (Syracuse Research Corp.) for his valuable help in searching the literature. Special thanks go to Melissa A. Strafford (*p*ION) for expertly assisting with some of the experiments.

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