

SUPPORTING INFORMATION

Titration in KCl/Water-Saturated Octanol: A Method for Quantifying Factors Influencing Ion-Pair Partitioning.

Robert A. Scherrer[†] and Stephen F. Donovan[‡]*

3M Pharmaceuticals, 3M Center, St. Paul, Minnesota 55144; BOPKa, White Bear Lake, Minnesota 55110, and The Pennsylvania Department of Health, Bureau of Laboratories, Exton, Pennsylvania, 19341-0500

Abstract for SI

The SUPPORTING INFORMATION contains a detailed description of the titration procedure and the method for calculating pK_a 's. Details for setting up the spreadsheet for the pK_a 's calculations are also included. There is a table of experimental pK_a 's values for water in octanol, a table of recommended titrator parameter settings, and a spreadsheet for the titration of pyridine-2-acetic acid hydrochloride. There is also a figure comparing the titration curve of magnesium salicylate with the theoretical curves based on the assumption of one or two pK_a 's values.

Table S1. The pK_a 's of water in KCl/water-saturated octanol by titration with KOH or HCl in ethanol. Calculations were made using points below pH 3.5 or above pH 12.0.

addition/titrant	pK_a 's \pm (SE of fit)	pH range (no. of pts)
HCl/KOH	-1.293 ± 0.003	2.2 – 3.5 (13)
CF ₃ SO ₃ H/KOH	-1.304 ± 0.003	1.5 – 3.5 (14)
CF ₃ SO ₃ H/KOH	-1.236 ± 0.006	1.4 – 3.5 (15)
0.01 mL KOH/HCl	-1.389 ± 0.006	1.8 – 3.5 (15)
none/HCl	-1.212 ± 0.005	2.0 – 3.5 (9)
octylamine/HCl	-1.336 ± 0.009	1.8 – 3.5 (7)
Av: -1.30 ± 0.06		
HCl/KOH	16.965 ± 0.003	12.0 – 13.4 (16)
HCl/KOH	17.070 ± 0.007	12.0 – 13.8 (9)
HCl/KOH	16.936 ± 0.004	12.0 – 14.0 (13)
CF ₃ SO ₃ H/KOH	16.926 ± 0.006	12.0 – 13.8 (10)
CF ₃ SO ₃ H/KOH	16.905 ± 0.010	12.0 – 14.0 (13)
none/KOH	17.046 ± 0.008	12.0 – 14.0 (10)
none/KOH	17.047 ± 0.006	12.0 – 14.0 (9)
diphenylphosphate/KOH	17.030 ± 0.002	12.0 – 14.0 (8)
diphenylphosphate/KOH	17.064 ± 0.002	12.0 – 14.0 (8)
Av: 17.00 ± 0.07		

SUPPORTING EXPERIMENTAL SECTION

This section provides a detailed description of a titration in octanol and the calculation of pK_a values. It duplicates some of the EXPERIMENTAL of the main article, but is repeated here to provide a single source for all of the information.

Equipment and Materials. The GLpKa model titrator was obtained from Sirius Analytical Instruments Ltd., East Sussex, UK (US offices: Sirius Analytical Inc., Piscataway, NJ 08854). It is equipped with a 50-well autosampler with temperature-controlled tray, a large 25 mL burette for dispensing saline, and five 5-mL precision dispensers capable of delivering as low as 0.00042 mL of titrant. A compact probe unit combines the electrode, stirrer, temperature probe, capillary dispenser tubes and tubing for inert gas. The titrations take place in 25-mL flat-bottomed titration vials (“Sirius vials”). The Sirius electrode is a Ag/AgCl reference half-cell electrode made in the UK. It is quality tested and designed to fit the probe unit. The temperature during each titration is recorded to a tenth of a degree C, but it was difficult to maintain a specific temperature. A redesigned autosampler tray introduced by Sirius in 2006 provides improved channeling to allow better temperature control. A blanket of argon protects the vial contents from CO₂ during the titration. The octanol is ACS spectrophotometric grade, $\geq 99\%$ from Aldrich.

Preparation of Titrants. The 0.5 N titrants were prepared using DILUT-IT analytical concentrates (J. T. Baker), designed for the preparation of 1.00 L solutions of 1.00 N KOH and HCl. By diluting each, instead, with USP absolute ethanol to 2.00 L in volumetric flasks (nitrogen atmosphere, concentrate added to ethanol), 0.5 N solutions were obtained. The water content was calculated by the difference in weight of the ampoule contents and the weight of 1.00 mole of titrant. The final KOH contained 150.9 g of water (7.54 % v/v), and the HCl, 123.3 g of water (6.16 % v/v). Standardizations were carried out in the usual manner in water. The titrants are stored in Sirius-prescribed

polypropylene containers fitted with polypropylene Luer lock connectors and the KOH is protected with a soda-lime tube.

Caution: The long-term stability of the glass components of the burette to 0.5 N KOH in ethanol is unknown. No problems or changes were observed with the Sirius burette, in which it was routine practice to use the ethanolic titrants for one-week periods. In earlier work, a burette of different design and make was rendered useless by prolonged standing with 0.1 N NaOH in octanol/methanol (3:1) containing 4% water.

Preparation of KCl/Water-Saturated Octanol. Precautions are taken throughout to avoid absorption of CO₂ from the air. The octanol medium is prepared by adding 50.0 mL of 0.15 M KCl (in highly purified and degassed water), to a freshly opened 2 L bottle of octanol, under nitrogen. The mixture is agitated with venting, and then stirred magnetically overnight in the closed bottle. Over time, KCl crystallizes from the approximately 2 mL of remaining aqueous phase. The octanol is transferred, as needed, by nitrogen pressure to a dispensing bottle fitted with a calibrated syringe and protected with a soda lime tube.

Titration Setup for Titrations in Octanol. The reservoirs for the aqueous and ethanolic titrants are exchanged at their Luer lock fittings. The 5-ml dispensers are thoroughly flushed with the new titrants. (Changing from aqueous to ethanolic titrants requires using the automated debubbler process to remove the last of the denser aqueous titrant) The electrode rinse flask is filled with 50% ethanol, and fresh pH 7.00 buffer is put in the number 1 position in the tray. The machine is now mechanically ready for use.

Preparation of the sample. A weighed sample is dissolved in 15.00 mL of KCl/water-saturated octanol (usually added in 5.00 mL increments) in a 25 mL Sirius vial. Sonication while stirring with a spatula is recommended, regardless of how easily the sample appears to dissolve. Warming in a water bath is often necessary. The solutions should be checked again just prior to starting a series of titrations. Up to 16 samples have been run overnight.

A list of relevant parameter values for these titrations is provided in Table S2. A major difference from aqueous titrations is a less strict electrode equilibration requirement, 0.010 Δ pH/min instead of 0.001 Δ pH/min, and a longer slug delay time of 3 to 10 sec instead of 1 sec. A feature of the titrator program is that the stirrer stops before reading each point while waiting for the electrode stability requirements to be met.

TABLE S2. Titration Parameters. The selected titration method is Aqueous Titration for pK_a . The sample is presented as a given molar solution at the final volume so the titrator dispenses no water.

assay type	sample
technique	pK_a
initial "water" volume	15.00 mL
initial pH adjustment	none
minimum volume addition	0.000420 mL
maximum volume addition	0.010000 mL
delta pH step	0.100 to 0.150 Δ pH
drift increase allowed	yes (in the stability criteria)
seconds per pH	1
required stability criteria	0.01000 Δ pH/min
stability point collected every	1.00 sec
post dispense delay time	10 sec
stability point timeout after	60 sec (minimum time, 12 sec)
slug delay time	3 to 10 sec (usually 5)
delay before point collect	1 sec

The BOpKCalc Program. A master spreadsheet is setup in the pattern shown in Figure 4. Columns D and E are set to contain the pH and volume data points from the titration. Points are trimmed from columns D and E as required. Removal from the bottom of the column presents no problem, but removal from the top requires adjustments to formulas, described later. Column F contains the calculated volume of titrant for each point determined by the formula using data in columns G to K. The Solver program will try to minimize the difference between column F and the experimental values in column E. Columns G, H and I hold the calculated titrant volumes for the ionizable groups pK_{a1} to pK_{a3} , (cells B5 to B7) based on their pK_a 's and corresponding equivalent volumes (cells C5 to C7). The slope correction in column J is usually set at 0.00080 mL/pH. Column K contains the sum of the values in columns G thru J. Columns N and O contain the full range of observed volume and pH data points pasted from the pKaLOGP data files.

Equations for Calculation of pK_a 's:

$$F3 = B4 + K3$$

$$G3 = ((C5) * 10^{(D3 - B5)}) / (1 + 10^{(D3 - B5)})$$

$$H3 = ((C6) * 10^{(D3 - B6)}) / (1 + 10^{(D3 - B6)})$$

$$I3 = ((C7) * 10^{(D3 - B7)}) / (1 + 10^{(D3 - B7)})$$

J3 = (D3 - D3) * B8 [Note: if data points are removed from the top of columns D and E, say to row 5, the formula in J5 should be changed to = (D5 - D5) * B8 and copied down to row J65, and from row J75 to J151]

$$K3 = \text{SUM}(G3:J3)$$

The above formulas are copied to rows 4 to 65.

B10 =SUMXMY2(E3:E65,F3:F65)

B12 =RSQ(E3:E65,F3:F65)

B13 =CORREL(E3:E65,F3:F65)

B14 =COVAR(E3:E65,F3:F65)

Experimental Volume and pH values are transferred from the data sheet to columns N and O, beginning at row 3, from where they are copied to the proper D and E columns. The latter set is trimmed as desired. (Reminder: If data points are removed from the top of columns D and E, the formula for column J should be changed as indicated above.)

Not visible are the cells used to calculate and draw the theoretical titration curve based on the values in cells B4 to C8. These cells are found in extensions of columns D thru L, rows 75 to 151.

D75:D151 An increasing sequence of pH values from -2.00 to 17.00 in 0.25 unit steps

E75:E151 Blank, no entry in the volume column

F75 =B\$4+K75

G75 =((C\$5)*10^(D75 - B\$5))/(1+10^(D75 - B\$5))

H75 =((C\$6)*10^(D75 - B\$6))/(1+10^(D75 - B\$6))

I75 =((C\$7)*10^(D75 - B\$7))/(1+10^(D75-B\$7))

J75 =(D75-D\$3)*B\$8

K75 =SUM(G75:J75)

The formulas for F75 thru K75 are copied to rows 76 to 151.

A plot of pH vs Volume is overlaid on the spreadsheet to display the data points and calculated curve. The calculation uses the Solver program from Microsoft to find the best fit of the calculated curve to the data points. In the simple situation here, the pK_a , the equivalent volume and the baseline offset are iteratively varied to minimize the sum of the differences squared. The statistics in cells B12 to B14 are provided by Excel. (The standard deviation can be added to this list.) The standard deviation and standard error are determined using a macro written by E.J. Billo and available on a disk or CD accompanying one of the two editions of his book, *Excel for Chemists*.²⁷ When the macro is open, it is accessed in the Tools dropdown menu of Excel. Two other references were suggested by a referee. They are: S1) de Levie, *How to use Excel in Analytical Chemistry*, Cambridge University Press, and S2) Howarth et al. *Tetrahedron Lett.* **2007**, *48*, 3337 on using Excel for analyzing acid base titrations and log P analyses.

Step-by-Step Calculation of the pK_a of Benzoic Acid. The pKaLOGP program that accompanies the Sirius titrator is opened and the desired benzoic acid .SDS file retrieved. Dropdown menus in the program provide access to the Raw Data and Settings files. Right-clicking in the window with the titration curve brings up a list of options. Select “Export as x,y data to file.” The file is saved as a .TXT file in the pKaLOGP folder. It contains a list of volume and pH values, in this case, 43 points from pH 5.7 to 14. The two columns of data should be copied and pasted to one side of the spreadsheet (at N3) for storage and reference and then copied to the working portion of the spreadsheet, columns D and E. A full plot of the titration curve appears in the overlaid chart set up for this purpose. In this example, since we are interested in the pK_a of benzoic acid and not the pK_a of water, the pH values above pH 11 in column D, and the corresponding volume terms in E, are deleted.

The pH and Volume scales on the x- and y-axes of the plot should also be adjusted. Next, estimated values for the pK_a and the equivalent volume are placed in C5 and B5. These are taken from the experimental curve. The equivalent volume appears to be about 0.17 mL and the pK_a is taken as the pH at the midpoint of the curve, about 7.7. The estimated baseline offset is entered in B4 as 0. This produces a calculated curve, which is a fine match for starting the next step using Solver.

“Solver” is opened from the Tools menu. A Solver window with query boxes appears. Set the target cell as \$B\$10. Select the button “Equal to Min.” The object is to minimize the least-squares difference between the experimental and calculated curves, which is the value found in the target cell. Fill in the “cells to change” by clicking on cells with variable terms while holding the control key, or type \$B4:\$B5, \$C5. Click the Solve button. After a brief moment a new window appears. Click the “Save the Solution” button, and then “OK”.

The pK_a obtained is 7.573. To make these calculations consistent and comparable, only points within 2 pH units of the pK_a are used. Therefore, the points above pH 9.57 are removed and the calculation repeated. The new result is pK_a 7.576, equivalent volume 0.170 and Baseline offset, -0.003. The values for R^2 , the correlation and the covariance are calculated by Excel (B12:B14). The standard deviation (STDEV) is also available in Excel.

Additional statistics are provided by the macro, Solvstat.xls,^{27b} which temporarily inserts into the Tool menu when it is opened. On the Tools menu, the heading “Solver statistics ...” is clicked to bring up a series of four windows covering four steps.

Step 1: Select range of known y's. Drag the cursor over the experimental volumes, \$E\$3:\$E\$27. Click OK.

Step 2: Select range of calculated y's. Drag the cursor over the calculated volumes, \$F\$3:\$F\$27. Click OK.

Step 3: Select the cells containing optimized terms obtained by using the solver. (Select at least two.) \$B\$5:\$C\$5. Click OK.

Step 4: Select a 3 row by 2 column area for results. \$B\$22:\$C\$24. The pK_a is 7.576, the standard deviation of pK_a is 0.0027 and the standard error is 0.0006. The raw data file gives the average assay temperature as 26.1° C.

Multiple pK_a Calculations for Pyridine-2-acetic Acid Hydrochloride, 19, 30. Examination of the titration curve, Figure S1, brings alternative strategies for calculating the pK_a values and equivalent volumes. The first pK_a comes at a pH where protonation of water will be competing, so the pK_a of water must be included, as a fixed value or by simultaneous solution. The latter is preferred in this case because of the large number of data points in the range below pH 3.5. The equivalent volumes of both ionizable groups should be identical, but the second ionization is more clearly defined. The formula for the first equivalent volume in cell C5 is set to “=C6”. The easiest solution is to solve for the five variables: the baseline offset, the first and second pK_a , the water pK_a and the second equivalent volume. The calculated pK_a of water is -1.215 ± 0.005 , comparing reasonably well with the average value of -1.30 .

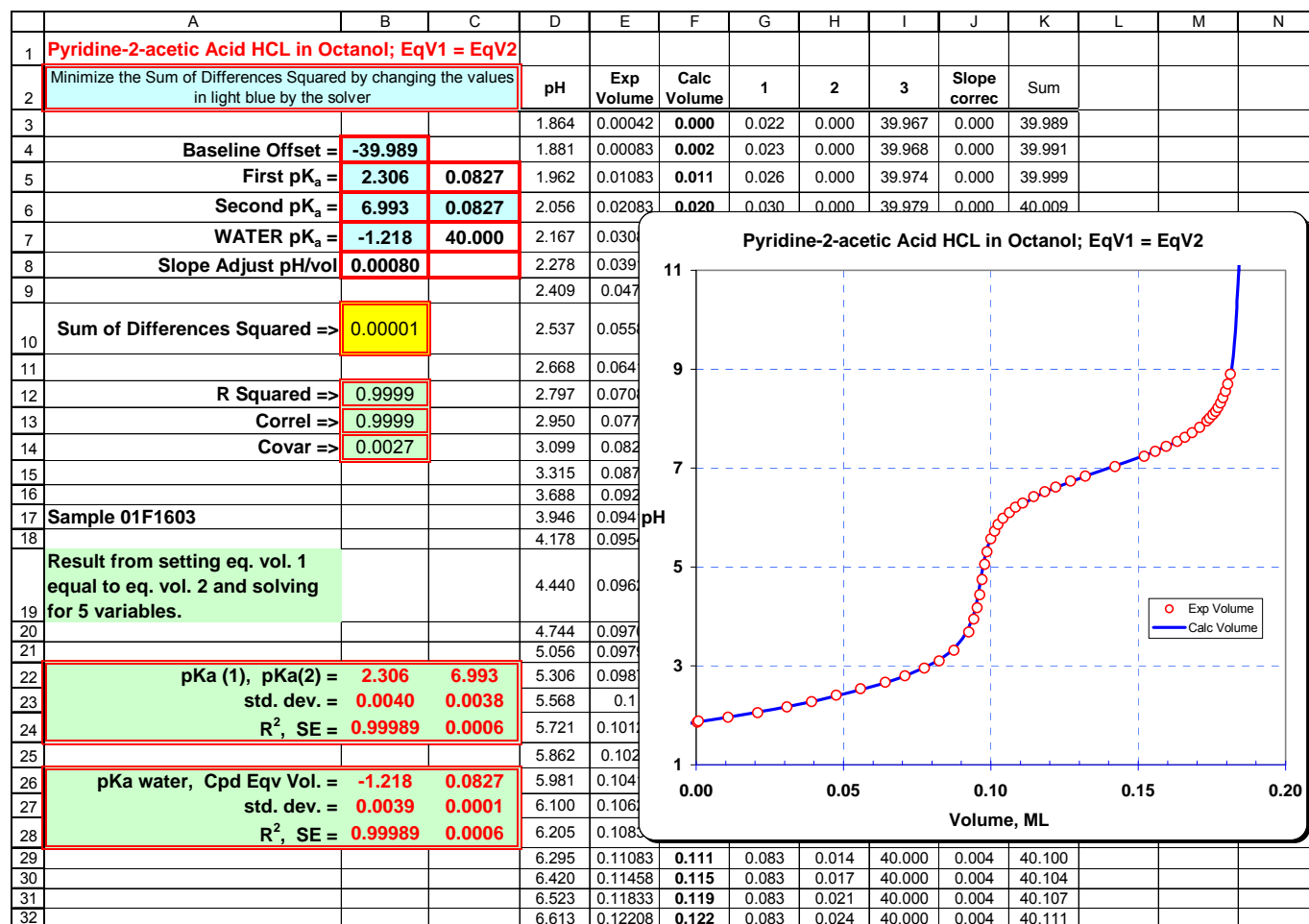


Figure S1. Spreadsheet for the determination of two pK_a” values for pyridine-2-acetic acid hydrochloride and the pK_a” of water.

The calculation method is robust. An alternative stepwise procedure gives essentially the same values. This involves first solving for the equivalent volume and pK_a” for the pyridine ionization. Then that equivalent volume is used for the first ionization constant while also solving simultaneously for the ionization constant of water. The differences are that the equivalent volume is 0.0830 instead of 0.0827; pK_a” values are 6.983 (0.01 lower) and 2.325 (0.02 higher) and the calculated water pK_a” values are identical.

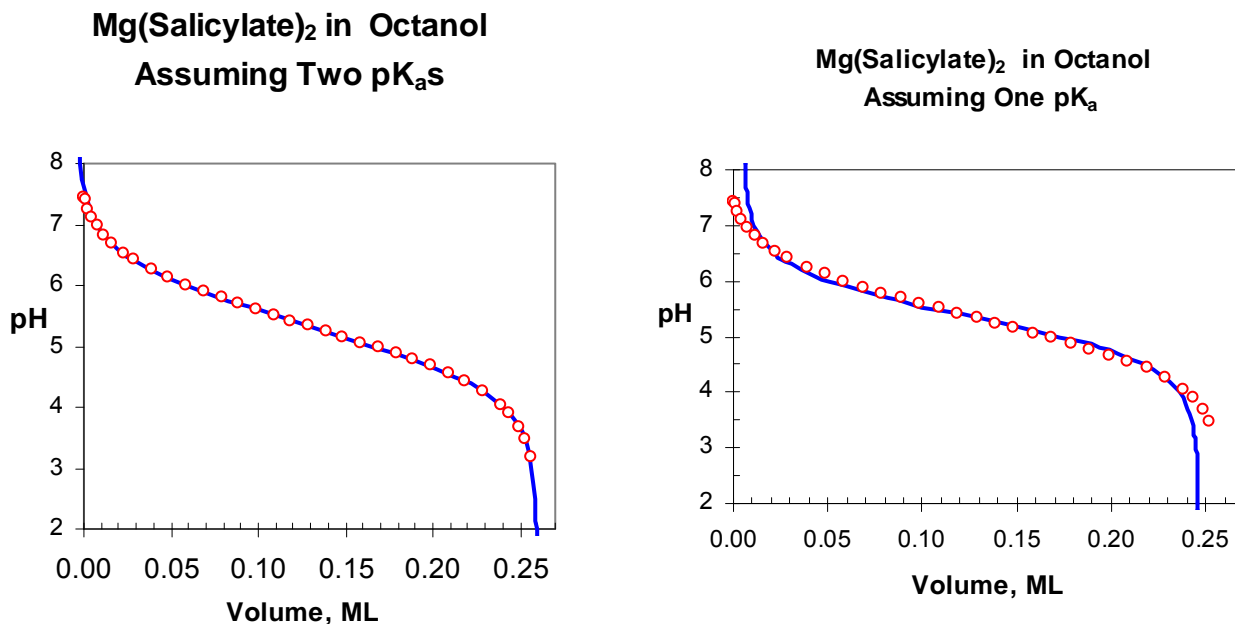


Figure S2. Calculated and experimental titration curves for magnesium salicylate showing the distinct formation of a chloromagnesium salicylate intermediate (**31a** and **31b**, Chart 2). (a) Calculated for two ionization constants: pK_{a1} : 4.862 ± 0.003 , R^2 1.0000, SE 0.0006. pK_{a2} : 5.815 ± 0.003 , R^2 1.0000, SE 0.0006. (b) Calculated for a single pK_a on the assumption of a dissociated and highly mobile ionized system: pK_a : 5.36 ± 0.01 , $R^2 = 0.9954$, SE 0.0059. Titration time: 32 min to pH 2.4.