Pharmaceutical Research, Vol. 14, No. 3, 1997 Report

Theory and Computer Programs for Calculating Solution pH, Buffer Formula, and Buffer Capacity for Multiple Component System at a Given Ionic Strength and Temperature

Hirokazu Okamoto,[1,2] Kiyoko Mori,[1] Kumiko Ohtsuka,[1] Hiroyuki Ohuchi,[1] and Hiroaki Ishii[1]

Received December 2, 1996; accepted December 11, 1996

Purpose. A theory and computer programs running on Microsoft® Excel for Windows for calculation of solution pH, buffer formula, and buffer capacity at a given ionic strength and temperature were developed. The theory does not limit the category of buffer components, the number of buffer components, or the number of ionizations for each buffer component. The usefulness of the programs was examined.

Methods. The formulas for 7 single component buffer solutions and 2 multiple component buffer solutions composed of citrate, phosphate, Tris, borate, and glycine were calculated. The solution pH values were measured at 25, 40, 55 and 70°C for comparison with the calculated pH values.

Results. Of the 108 predictions made, 96 were of pH values within ± 0.1 pH unit of the measured values, at temperatures ranging from 25°C to 70°C and at ionic strengths ranging from 0.1 M to 0.5 M.

Conclusions. These programs will be useful for identifying appropriate buffer solutions at various temperatures and/or ionic strengths.

KEY WORDS: pH; buffer solution; ionic strength; computer program.

INTRODUCTION

In the early stage of developing a candidate compound as a new drug, its solubility and stability in aqueous solutions are measured as a function of solution pH. To estimate solution stability at room temperature or lower temperature, stability studies are often run at elevated temperatures. Since the ionic strength of the solution may affect the solubility and degradation rate, the ionic strength should be set at a predetermined value. Computer programs for calculating solution pH, buffer formula, and buffer capacity will help us identify the appropriate buffer solutions to be used.

There have been many reports on methods and/or computer programs for calculation of solution pH, buffer formula, and/or buffer capacity (1–4). However, these are somewhat inconvenient for use in designing a buffer solution, since they ignore effects of ionic strength, do not involve effects of temperature, treat acids, bases, and/or ampholytes separately, do not report the buffer formula (ingredient weights and/or stock solution volume), and/or do not run on Windows®, which is now widely used at workplaces.

We developed computer programs to calculate solution pH, buffer formula, and buffer capacity at a given ionic strength and temperature using Excel on Windows®. The theory placed no limitations on the categories of buffer components (acid, base, or ampholyte), the number of buffer components, or the number of ionizations for each buffer component. While the programs can be used for up to 10 buffer component systems and up to 4 ionizations for each buffer component in order to prevent the programs from becoming too large.

THEORETICAL

Practical Dissociation Constant Complex and Practical Ion Product of Water

In this report "component" is defined as a substance having buffer effects (eg, acetate), "ingredient" as a chemical used to prepare a buffer solution (eg, acetic acid, sodium acetate, sodium chloride, hydrochloric acid, and sodium hydroxide), and "species" as a molecule or ion in a solution (eg, CH_3COOH, CH_3COO^-, Na^+, Cl^-, H^+, and OH^-). The concentrations of ingredients and species are denoted by C* and **C**, respectively. Below, H^+, OH^-, Na^+, Cl^-, HCl, NaOH, and NaCl are denoted by the subscripts h, oh, na, cl, hcl, naoh, and nacl, respectively.

We considered a buffer solution composed of M buffer components, HCl, NaOH, and/or NaCl. The counter ions of the buffer species were chloride or sodium. Each buffer component was a weak polyacidic base, weak polybasic acid, or ampholyte with m_i ionizations and yielded $m_i + 1$ species with different charges ($z_{i,j}$) in a solution.

$$z_{i,j} = n_i - j \tag{1}$$

where i means the i th buffer component (i = 1, 2, 3, ... M), j means the j th species (j = 0, 1, 2, 3, ... m_i), and n_i is the most positive charge that the i th buffer component can have in solution. In the case of glycine, for instance, $z_{i,0} = n_i = +1$ ($CH_2(NH_3^+)COOH$), $z_{i,1} = 0$ ($CH_2(NH_3^+)COO^-$), and $z_{i,2} = -1$ ($CH_2(NH_2)COO^-$).

The activity coefficient $\gamma_{i,j}$, which is the ratio of activity $A_{i,j}$ to concentration $C_{i,j}$, is estimated by the Debye-Hückel equation:

$$\gamma_{i,j} = \frac{A_{i,j}}{C_{i,j}} = 10^{-z_{i,j}^2 N} \tag{2}$$

N is a function of ionic strength of solution I (5):

$$N = P\frac{I^{0.5}}{1 + I^{0.5}} - QI \tag{3}$$

Q is a parameter introduced by Davies (5) and set by the user of the programs. P for water depends on the absolute temperature T and is estimated by:

$$P = 1.116 \times 10^{-8}T^3 - 6.021 \times 10^{-6}T^2 + 1.547 \times 10^{-3}T + 0.2872 \tag{4}$$

which was obtained by fitting the P values reported in reference (6) to a cubic equation for T using a non-linear least-squares program installed in SigmaPlot® based on the Marqurdt-Levenber method.

---

[1] Pharmacia & Upjohn, Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki 300-42, Japan.

[2] To whom correspondence should be addressed. (e-mail: hirokazu.okamoto@am.pnu.com)

When the dissociation constant $K_{0,i,j}$ at ionic strength $I_{0,i,j}$ and at absolute temperature $T_{0,i,j}$ is available, the thermodynamic dissociation constant $K_{i,j}$ at zero ionic strength and at given temperature T can be estimated by (1,7):

$$K_{i,j} = \frac{A_{i,j}A_h}{A_{i,j-1}} = K_{0,i,j}10^{2(n_i-j)N_{0,i,j}} \exp\left[\frac{\Delta H^0_{i,j}}{R}\left(\frac{1}{T_{0,i,j}} - \frac{1}{T}\right)\right] \tag{5}$$

where $\Delta H^0_{i,j}$ is the standard enthalpy of dissociation and R is the gas constant.

The practical dissociation constant $K'_{i,j}$ and the practical dissociation constant complex $k_{i,j}$, which is the product of the first j practical dissociation constants, are defined and calculated by:

$$K'_{i,j} = \frac{C_{i,j}C_h}{C_{i,j-1}} = K_{i,j}\frac{\gamma_{i,j-1}}{\gamma_{i,j}\gamma_h} = K_{i,j}10^{2(j-n_i)N} \tag{6}$$

$$k_{i,j} = \prod_{\lambda=1}^{j} K'_{i,\lambda} = \prod_{\lambda=1}^{j} K_{i,\lambda}10^{[j(j+1)-2jn_i]N} \tag{7}$$

Here, $k_{i,0}$ is defined to be unity.

The negative logarithm of the ion product of water pKw at absolute temperature T is estimated by:

$$\begin{aligned} pKw = -\log(A_hA_{oh}) &= -6.513 \times 10^{-7}T^3 \\ &+ 7.750 \times 10^{-4}T^2 - 0.3218T + 58.29 \end{aligned} \tag{8}$$

which was obtained by fitting the pKw values reported in reference (8) to a cubic equation for T.

The practical ion product of water Kw′ is calculated by:

$$Kw' = C_hC_{oh} = \frac{Kw}{\gamma_h\gamma_{oh}} = Kw10^{2N} \tag{9}$$

Computer Programs to Calculate Buffer Formula

The buffer formulas are calculated by entering the desired pH, total buffer concentrations, ionic strength, and temperature as follows.

The concentrations C_h, C_{oh}, and $C_{i,j}$ are calculated as ($C_{i,t}$ is the total concentration of the i th buffer component):

$$C_h = 10^{(-pH+N)}, \qquad C_{oh} = Kw'/C_h \tag{10}$$

$$C_{i,j} = C_{i,t}\frac{k_{i,j}C_h^{-j}}{\sum\limits_{j=0}^{m_i} k_{i,j}C_h^{-j}} \tag{11}$$

A factor for charge balance S and partial ionic strength I′ are calculated as:

$$S = C_{oh} - C_h - \sum_{i=1}^{M}\sum_{j=0}^{m_i}(n_i - j)C_{i,j} \tag{12}$$

$$I' = I - 0.5\left[C_h + C_{oh} + \sum_{i=1}^{M}\sum_{j=0}^{m_i}(n_i - j)^2C_{i,j}\right] \tag{13}$$

S equals $C_{na} - C_{cl}$ while I′ equals 0.5 ($C_{na} + C_{cl}$). In the case of $I' < 0.5|S|$, the ionic strength I should be increased by the user in order to increase I′.

When the system is composed of single buffer component, the concentrations of two ingredients, $C^*_{1,a}$ and $C^*_{1,b}$, are calculated by:

$$C^*_{1,a} = \frac{x_{1,b}S + y_{1,b}C_{1,t}}{x_{1,a}y_{1,b} - x_{1,b}y_{1,a}}, \qquad C^*_{1,b} = \frac{-x_{1,a}S - y_{1,a}C_{1,t}}{x_{1,a}y_{1,b} - x_{1,b}y_{1,a}} \tag{14}$$

where the parameter x is related to the number of species having buffer action in the molecular equation for the ingredient, i.e., x = 1 except when the ingredient is HCl or NaOH (x = 0). The parameter y is related to the number of Na or Cl in the molecular equation for the ingredient; for example, +1 for NH_4Cl and −2 for Na_2HPO_4.

When the system is composed of multiple buffer components, $C^*_{i,\lambda i}$ equals $C_{i,t}$, supposing that the λ_i th ingredient is used for the i th buffer component. For an acetic acid/trisodium phosphate system, for instance, $\lambda_1 = 0$ and $\lambda_2 = 3$, respectively. The concentration of NaOH or HCl to be added is calculated as:

$$U = S + \sum_{i=1}^{M} z_{i,\lambda_i}C^*_{i,\lambda_i} = S + \sum_{i=1}^{M} z_{i,\lambda_i}C_{i,t} \tag{15}$$

When U is positive, NaOH should be added, while if it is negative, HCl should be added.

The concentration of NaCl to be added to adjust the ionic strength is calculated as:

$$C^*_{nacl} = I' - 0.5(|U| + \sum_{i=1}^{M}\sum_{j=0}^{m_i}|z_{i,j}|C^*_{i,j}) \tag{16}$$

Computer Programs to Calculate Solution pH

The solution pHs are calculated by entering the desired buffer formula (concentrations of ingredients) as follows.

S and I′ are calculated as:

$$S = C^*_{naoh} - C^*_{hcl} - \sum_{i=1}^{M}\sum_{j=0}^{m_i}(n_i - j)C^*_{i,j} \tag{17}$$

$$I' = C^*_{nacl} + 0.5\left[C^*_{naoh} + C^*_{hcl} + \sum_{i=1}^{M}\sum_{j=0}^{m_i}|n_i - j|C^*_{i,j}\right] \tag{18}$$

The initial $k_{i,j}$ and Kw′ are calculated with initial I = 10 M. The C_h value is determined by applying the Newton-Raphson method to Eq. (19) (the initial $C_h = 1 \times 10^{-15}$ M):

$$f(C_h) = C_h + S - \frac{Kw'}{C_h} + \sum_{i=1}^{M} C_{i,t}\omega_i\alpha_i^{-1} \tag{19}$$

where:

$$\alpha_i = \sum_{j=0}^{m_i} k_{i,j}C_h^{-j}, \qquad \omega_i = \sum_{j=0}^{m_i}(n_i - j)k_{i,j}C_h^{-j} \tag{20}$$

The programs repeat this calculation 24 times.

After calculating $C_{i,j}$ with Eq. (11), the I value is calculate as:

$$I = 0.5\left[C_h + C_{oh} + C_{na} + C_{cl} + \sum_{i=1}^{M}\sum_{j=0}^{m_i}(n_i - j)^2C_{i,j}\right] \tag{21}$$

Using the I value, $k_{i,j}$, Kw′, and C_h values are recalculated. The calculations above are repeated 7 times to obtain satisfactory C_h and I values.

Calculation of Buffer Capacity

Buffer capacity (β) is calculated as the ratio of an increment of strong base (or acid) to a small change in pH brought about by this addition (9):

$$\beta = \frac{dC_{na}}{dpH} = -2.303C_h\frac{dC_{na}}{dC_h}$$

$$= 2.303\left[\sum_{i=1}^{M} C_{i,t}C_h(\omega_i'\alpha_i - \omega_i\alpha_i')\alpha_i^{-2} + C_h + C_{oh}\right] \quad (22)$$

where $\alpha' = d\alpha/dC_h$ and $\omega' = d\omega/dC_h$.

MATERIALS AND METHODS

Materials

The operating system Microsoft® Windows™ (version 3.1, Microsoft, Inc.), Microsoft® Excel for Windows (version 5.0, Microsoft, Inc.), and scientific graph preparation software SigmaPlot® (version 2.0, Jandel Co.) were installed in a personal computer Cerebris 590 (Digital Equipment Co.) with a Pentium CPU (90 MHz, Intel Co.).

The chemicals used to prepare buffer solutions were citric acid, anhydrous (lot PTK0027), sodium phosphate, monobasic, dihydrate (lot PTG3796), boric acid (lot ESF6505), glycine (lot PTR1027), sodium chloride (lot 6161), 1N hydrochloric acid (lot SKQ7820), and 1N sodium hydroxide solution (lot CAF7423) purchased from Wako Pure Chemical Industries, Ltd. (Osaka), and Trizma® base (Tris, lot 32H5619) purchased from Sigma Chemical Co. (St. Louis). Deionized water was prepared using the Milli-Q SP TOC (Japan Millipore Ltd., Tokyo).

pH Measurement

Single-component and multiple-component buffer solutions were prepared using the formulas calculated by the programs. After measuring the solution pH at 25°C with a pH meter (F-22, Horiba, Kyoto), the solution temperature was raised to 40, 55, and 70°C to measure the solution pH. The actual temperatures ranged from 23.1 to 25.2°C, 39.0 to 41.4°C, 54.0 to 55.2°C, and 69.0 to 70.7°C, respectively. The pH meter was calibrated with proper standard buffer solutions at the specific temperature for measurement. The electrode used was an Ag/AgCl combination electrode 6378-10D (Horiba) comprised of a glass electrode, reference electrode, and temperature compensation electrode in one body. The temperature of the solution was the output on the display of the F-22 pH meter.

RESULTS AND DISCUSSION

The buffer solutions prepared were 0.01 M citrate at pHs 4, 5, and 6, 0.01 M phosphate at pH 7, 0.01 M Tris at pH 8, 0.01 M borate at pH 9, 0.01 M glycine at pH 10, and multiple (five) component systems at pHs 6 and 9 at ionic strengths of 0.1, 0.3, and 0.5 M. The dissociation constants and standard enthalpies used are listed in Table I (7). The pH values of the given formulas at 25, 40, 55, and 70°C and ionic strengths of 0.1, 0.3 and 0.5 M were calculated by the programs with a Q value of 0 or 0.1. Figure 1 illustrates the ΔpH (= "pH measured" − "pH calculated") for these solutions. The measured pH values of 0.01 M Tris and 0.01 M glycine decreased by more than 1 pH unit when temperature was increased from 25°C to 70°C (data not shown) because of the high enthalpies (Table I) (1). The solution pHs were well predicted when Q = 0. Of the 108 ΔpHs, only 12 exceeded a difference of ±0.10 at Q = 0, while 43 exceeded a difference of ±0.10 at Q = 0.1. Davies proposed the use of Q for better prediction of the effect of ionic strength on the activity coefficient (5). However, the pHs calculated agreed well with the measured values when Q = 0 at ionic strength up to 0.5 M in this study. Since Q is an empirical value and has no theoretical background, some buffer systems may need to input a Q value.

Equation (2) predicts that neutral species ($z_{i,j} = 0$) have activity coefficients equal to 1. However, if the neutral species

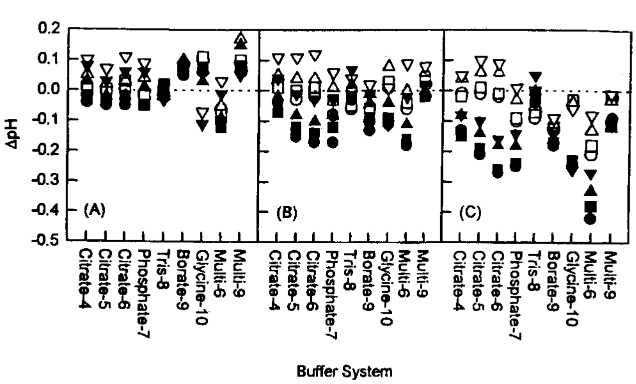

Fig. 1. ΔpH (= "pH measured" − "pH calculated") at 25, 40, 55, and 70°C at ionic strengths of 0.1 M (A), 0.3 M (B), and 0.5 M (C). Solution pH was measured at 25°C (○, ●), 40°C (□, ■), 55°C (△, ▲), or 70°C (▽, ▼). The open symbols are ΔpHs when pH was calculate for Q = 0, while the closed symbols are those for Q = 0.1. The compositions of the 1-L solutions are as following. Three values of 1N-NaOH, 1.004N-HCl, and sodium chloride are those for I = 0.1 M, I = 0.3 M, and I = 0.5 M solutions, respectively. Citrate-4 (0.01 M citrate at pH 4): Citric acid 1.921 g; 1N-NaOH 11.78 mL, 12.47 mL, 12.68 mL; sodium chloride 4.993 g, 16.60 g, 28.27 g. Citrate-5 (0.01 M citrate at pH 5): Citric acid 1.921 g; 1N-NaOH 19.11 mL, 20.11 mL, 20.42 mL; sodium chloride 4.132 g, 15.67 g, 27.31 g. Citrate-6 (0.01 M citrate at pH 6): Citric acid 1.921 g; 1N-NaOH 25.77 mL, 26.83 mL, 27.10 mL; sodium chloride 3.073 g, 14.58 g, 26.22 g. Phosphate-7 (0.01 M phosphate at pH 7): Sodium phosphate, monobasic, dihydrate 1.560 g; 1N-NaOH 5.793 mL, 6.414 mL, 6.582 mL; sodium chloride 4.583 g, 16.20 g, 27.87 g. Tris-8 (0.01 M Tris at pH 8): Tris 1.211 g; 1.004N-HCl 6.025 mL, 6.231 mL, 6.288 mL; sodium chloride 5.490 g, 17.17 g, 28.85 g. Borate-9 (0.01 M borate at pH 9): Boric acid 0.6183 g; 1N-NaOH, 4.306 mL, 4.522 mL, 4.583 mL; sodium chloride, 5.592 g, 17.27 g, 28.95 g. Glycine-10 (0.01 M glycine at pH 10): Glycine 0.7507 g; 1N-NaOH 6.807 mL, 6.684 mL, 6.405 mL; sodium chloride, 5.446 g, 17.14 g, 28.85 g. Multi-6 (multiple component system at pH 6): Citric acid 1.921 g; sodium phosphate, monobasic, dihydrate 1.560 g; Tris 1.211 g; boric acid 0.6183 g; glycine 0.7507 g; 1N-NaOH 17.05 mL, 18.41 mL, 18.78 mL; sodium chloride 2.347 g, 13.82 g, 43.54 g. Multi-9 (multiple component system at pH 9): Citric acid 1.921 g; sodium phosphate, monobasic, dihydrate 1.560 g; Tris 1.211 g; boric acid 0.6183 g; glycine 0.7507 g; 1N-NaOH 44.59 mL, 44.64 mL, 44.52 mL; sodium chloride 0.2421 g, 11.92 g, 23.61 g.

Table I. Dissociation Constants and Standard Enthalpies at 25°C for Buffer Components[a]

	Dissociation constant			Standard enthalpy, cal/mole		
Component	pK_1	pK_2	pK_3	for K_1	for K_2	for K_3
Borate	9.236	—	—	3400	—	—
Citrate	3.128	4.761	6.396	980	530	−780
Glycine	2.350	9.778	—	1000	10600	—
Phosphate	2.148	7.198	12.375	−1900	900	3800
Tris	8.073	—	—	11360	—	—

[a] Reported in R. M. Smith and A. E. Martell *Critical stability constants. Volume 6: Second Supplement*, Plenum Press, New York, (1989).

is a zwitterion, it will have a large dipole moment and the expression for its activity coefficient may have to contain a term K_r, a "salting in" constant (8):

$$\gamma_{+-} = 10^{-K_r I} \tag{23}$$

The "salting in" constant is reportedly about 0.32 for alpha-amino acids and about 0.6 for dipeptides in water (8). We measured the pHs of glycine, alanine, lysine, and glycylalanine buffer solutions and compared them to the calculated values (data not shown). Use of K_r values did not improve the prediction.

We assumed that the standard enthalpy for dissociation is a constant and independent of ionic strength and temperature. Although this assumption does not appear to result in a large error in calculation, the user should remember that increasing ionic strength and/or temperature may increase errors in calculation (1,7). We recommend that the user measures the solution pHs at designated temperatures to ensure that the programs calculate acceptable pH values.

In the early stage of developing a candidate compound as a new drug, its solubility and stability in aqueous solutions are measured using buffer solutions at various temperatures and/or ionic strengths. The computer programs we have developed help identify the buffer solutions appropriate for use.

REFERENCES

1. J. E. Kipp and D. F. Schuck. *J. Pharm. Sci.* **84**:1347–1352 (1995).
2. D. A. Ventura and H. Y. Ando. *J. Pharm. Sci.* **69**:891–896 (1980).
3. J. E. Kipp. *J. Chem. Educ.* **71**:119–121 (1994).
4. C. Mongay and V. Cerdá. *Comput. Chem.* **8**:213–216 (1984).
5. C. W. Davies. *J. Chem. Soc.* 2093–2098 (1938).
6. A. F. Martin. *Physical Pharmacy. 4th ed.* Lea & Febiger, Philadelphia, 1993, pp. 125–142.
7. R. M. Smith and A. E. Martell. *Critical stability constants. Volume 6: Second Supplement*, Plenum Press, New York, 1989.
8. A. F. Martin. *Physical Pharmacy. 4th ed.* Lea & Febiger, Philadelphia, 1993, pp. 143–168.
9. M. Koppel and K. Spiro. *Biochem. Z.* **65**:409–439 (1914)./A. Roos and W. F. Boron. *Resp. Physiol.* **40**:1–32 (1980)./D. D. Van Slyke. *J. Biol. Chem.* **52**:525–570 (1922).