Calculation of the equilibrium pH in a multiple-buffered aqueous solution based on partitioning of proton buffering: a new predictive formula

Minhtri K. Nguyen, Liyo Kao, and Ira Kurtz

Department of Medicine, University of California, Los Angeles, California

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Nguyen MK, Kao L, Kurtz I. Calculation of the equilibrium pH in a multiple-buffered aqueous solution based on partitioning of proton buffering: a new predictive formula. Am J Physiol Renal Physiol 296: F1521–F1529, 2009. First published April 1, 2009; doi:10.1152/ajprenal.90651.2008.—Upon the addition of protons to an aqueous solution containing multiple buffers, the final $H^+$ concentration ($[H^+]_{equil}$) at equilibrium is determined by the partitioning of added $H^+$ among the various buffer components. In the analysis of acid-base chemistry, the Henderson-Hasselbalch equation and the Stewart strong ion formulation can only describe (rather than predict) the equilibrium pH following a proton load since these formulas calculate the equilibrium pH only when the reactant concentrations at equilibrium are already known. In this regard, it is simpler to directly measure the equilibrium pH rather than measure the equilibrium reactant concentrations to calculate the equilibrium pH. As these formulas cannot predict the final equilibrium $[H^+]_{equil}$ following a proton load to a multiple-buffered aqueous solution, we developed a new quantitative approach for predicting the equilibrium $[H^+]_{equil}$ that is based on the pre-equilibrium concentrations of all buffers in an aqueous solution. The mathematical model used to derive our equation is based on proton transfer buffer equilibria without requiring the incorporation of electroneutrality considerations. The model consists of a quartic polynomial equation that is derived based solely on the partitioning of $H^+$ among the various buffer components. We tested the accuracy of the model using aqueous solutions with various buffers and measured the equilibrium pH values following the addition of HCl. Our results confirmed the accuracy of our new equation ($r^2 = 1$; measured pH vs. predicted pH), indicating that it quantitatively accounts for the underlying acid-base phenomenology.

 acid-base chemistry; proton; bicarbonate; Stewart; Henderson-Hasselbalch

THE ANALYSIS OF ACID-BASE reactions is important in the fields of chemistry, physiology, and clinical medicine. Although chemists continue to utilize the Henderson-Hasselbalch equation (5, 6) to model acid-base reactions macroscopically, the Stewart strong ion approach (8, 12–14) has been thought by some investigators to be a superior mechanistic approach to characterizing acid-base disorders in the clinical setting. One of the motivations for utilizing the Stewart equation is its purported superiority in modeling acid-base reactions in solutions with multiple buffers that more accurately reflects fluid compartments in vivo. The Henderson-Hasselbalch approach equates the equilibrium pH value to a single buffer pair that is typically bicarbonate based, given the importance of this buffer to extracellular body fluid buffering. This framework, however, fails to incorporate quantitatively the role of nonbicarbonate buffers such as phosphate, albumin, etc.

To correct this deficiency, Stewart derived a polynomial equation (simplified by Constable), which incorporates buffers in addition to bicarbonate (1–3, 12–14). Pivotal to the Stewart formulation is the purported role of electroneutrality in determining and modifying the $H^+$ concentration ($[H^+]$) of a solution. Although the principle of electroneutrality is central to the Stewart strong ion formulation, we have recently demonstrated that electroneutrality per se mechanistically does not dictate a specific $[H^+]$ in aqueous solutions (9). Most importantly, both the Henderson Hasselbalch and strong ion approaches are equilibrium models of acid-base balance. According to the isohydric principle, all buffers are in equilibrium with the same $[H^+]$ in a solution containing multiple buffers. Consequently, since the Henderson-Hasselbalch and strong ion approaches are equilibrium models, the isohydric principle underscores the fact that any buffer pair (assuming the $pK'$ is accurately known) can be utilized to calculate the equilibrium pH value, thereby negating the need quantitatively to incorporate multiple buffers and multiple $pK'$ values into a formula such as the Stewart equation to simply calculate the pH at equilibrium (9).

However, when one’s goal is to predict what the value of the equilibrium pH will be in a multiple-buffered aqueous solution following the addition of an acid, neither the Henderson-Hasselbalch nor the Stewart strong ion model can be used since their derivation models the behavior of the solution only when equilibrium has already been achieved.

In this article, we have derived a new mathematical model that accurately predicts quantitatively the partitioning of $H^+$ among various monoprotic3 buffer pairs in an aqueous solution and, moreover, can be utilized to predict the equilibrium $[H^+]_{equil}$ based on the pre-equilibrium reactant concentrations in a multiple-buffered solution. Our new equation was tested using various monoprotic buffer pairs to demonstrate its accuracy.

Derivation of the Predictive Multiple Buffer Equation

$$[H^+]_{equil} \times [OH^-]_{equil} = K_w \quad (Eq.1)$$

Let HA/A– and HB/B– represent two weak acid-conjugate base buffer pairs.

$$[H^+]_{equil} \times [A^-]_{equil} = K'_w[HA]_{equil} \quad (Eq.2)$$

3 The term “monoprotic” refers to buffer pairs that mediates single proton transfer reactions.

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\[
\frac{[H^+]}{[OH^-]} \times [B^-] = K_{eq}^\prime (HB)_{equal} \quad \text{(Eq. 3)}
\]

Let \(x\), \(y\), and \(z\) represent the quantity of \(H^+\) buffered by \(OH^-\), \(A^-\), and \(B^-\) buffers respectively.
Let suffix equilibrium = e
Let suffix initial = i
Therefore
\[
[H^+]_e = [H^+] - x - y - z \quad \text{(Eq. 4)}
\]
\[
[OH^-]_e = [OH^-] - x \quad \text{(Eq. 5)}
\]
\[
[A^-]_e = [A^-] - y \quad \text{(Eq. 6)}
\]
\[
[HA]_e = [HA] + y \quad \text{(Eq. 7)}
\]
\[
[B^-]_e = [B^-] - z \quad \text{(Eq. 8)}
\]
\[
[HB]_e = [HB] + z \quad \text{(Eq. 9)}
\]

At equilibrium, the \([H^+]\) is the same for all buffer pairs
\[
[H^+]_e = \frac{K_w}{[OH^-]_e} = \frac{K_i^0([HA], + y)}{[A^-]_e} = \frac{K_i^0([HB], + z)}{[B^-]_e} \quad \text{(Eq. 10)}
\]

Therefore
\[
[H^+]_e - x - y - z = \frac{K_w}{[OH^-]_e} - x = \frac{K_i^0([HA], + y)}{[A^-]_e} - y = \frac{K_i^0([HB], + z)}{[B^-]_e} - z = \frac{K_i^0([HA], + y)}{[A^-]_e} = \frac{K_i^0([HB], + z)}{[B^-]_e} \quad \text{(Eq. 11)}
\]

Since
\[
K_w = K_i^0([HA], + y)(K_i^0([HB], + z)) \quad \text{(Eq. 12)}
\]
\[
K_i^0([OH^-][HA]) + y(K_i^0([OH^-])) - x(K_i^0([HA])) - y(K_w)
\]
\[
y = \frac{-x(K_i^0([HA]) - K_i^0([HA])) - y(K_w)}{(x(K_i^0([HA])) - K_i^0([HA])) - y(K_w)} \quad \text{(Eq. 13)}
\]
\[
z = \frac{-x(K_i^0([HB]) - K_i^0([HB])) - y(K_w)}{(x(K_i^0([HB])) - K_i^0([HB])) - y(K_w)} \quad \text{(Eq. 14)}
\]

Since
\[
[H^+]_e - x - y - z = \frac{K_w}{[OH^-]_e} \quad \text{(Eq. 11)}
\]
\[
\cdot [H^+]_e - x([H^+] + [OH^-]) + x^2 - y([OH^-]) + xy = 0 \quad \text{(Eq. 15)}
\]

Equation 14 can be reexpressed as follows
Let
\[
y = \frac{C - x(K_i^0([HA]))}{x(K_i^0) + D} \quad \text{(Eq. 17)}
\]

Where
\[
C = K_i^0([OH^-][HA] - K_w[A^-] \quad \text{(Eq. 18)}
\]
+x(CF + DE - DF([H⁺] + [OH⁻]) + Kᵯᵣ(FG
+ F[HA][OH⁻] - E[OH⁻]) + Kᵯᵣ(FG + D[HB][OH⁻]
- C[OH⁻]))
+DFG - CF([OH⁻]) - DE([OH⁻]) = 0  \text{(Eq. 22)}

Solve Eq. 22 for “x” by finding the roots of a quartic equation using wxMaxima software.
Calculate “y” using Eq. 17.
Calculate “z” using Eq. 18.
Calculate [H⁺]ᵣ using Eq. 11

[H⁺]ᵣ = [H⁺]ᵣ - x - y - z = \frac{Kᵯᵣ}{[OH⁻]} - x
= \frac{Kᵯᵣ([HA] + y)}{[A⁻]} - y = \frac{Kᵯᵣ([HB] + z)}{[B⁻]} - z \text{  \text{(Eq. 11)}}

This quantitative approach can be expanded to account for as many buffers as necessary.
The apparent equilibrium constant Kᵣ is calculated based on the thermodynamic equilibrium constant K according to the following equation (4, 10)

pKᵣ = pK - 0.51 \sqrt{l} \text{  \text{(Eq. 23)}}

where l = ionic strength

I = \frac{1}{2} \sum cZ² \text{  \text{(Eq. 24)}}

where c is the molar concentration of each ion, and Z is its charge.

METHODS

Equations 17, 18, and 22 are first used to calculate the partitioning of H⁺ among the various buffer pairs. Equation 11, which will be named the Predictive Multiple Buffer Equation, is then employed to predict the equilibrium [H⁺] that is calculated based solely on the partitioning of H⁺ among these buffer components. The equilibrium pH is first estimated using the thermodynamic equilibrium constant K of each buffer. The thermodynamic equilibrium constant K is the equilibrium constant of each buffer when its reactants are expressed in terms of ionic activities. Using the thermodynamic equilibrium constant Kᵣ, the initial equilibrium pH is first estimated based on the partitioning of H⁺ among the different buffer pairs according to Eq. 11. The partitioning of H⁺ among the different buffer pairs is also used to predict the equilibrium anionic buffer concentrations according to Eqs. 5, 6, and 8. The estimated initial equilibrium [H⁺], predicted equilibrium anionic buffer concentrations, and chloride concentration of added HCl are used to estimate the ionic strength at equilibrium since ionic strength is a function of all ions at equilibrium. The predicted ionic strength at equilibrium is then utilized to determine the apparent equilibrium constant Kᵣ of each buffer, which is calculated according to Eq. 23 (4, 10). The apparent equilibrium constant Kᵣ is the equilibrium constant of each buffer when its reactants are expressed in molar concentrations. The final equilibrium pH is then recalculated using the apparent equilibrium constant Kᵣ since the reactant concentrations are expressed in molar concentrations (4).

In validating the Predictive Multiple Buffer Equation, we tested the model using aqueous solutions with various buffer pairs and measured the pH changes following the addition of HCl. Each multiple-buffered solution contained two different buffers in 20 ml of H₂O. The following buffers were used each at a concentration of 0.01 M: Na PIPES (Amresco, Solon, OH)/Na HEPES (Sigma, St. Louis, MO); Na PIPES/Na MES (Sigma); Na PIPES/Na acetate (Sigma); Na HEPES/Na MES; Na HEPES/Na acetate; and Na MES/Na acetate. Buffers were incubated at 25°C in a water bath (Thermo Fisher Scientific, Waltham, MA), and the temperature was monitored by a thermometer (Fisher Scientific, Chino, CA) during the experiment. An initial equilibrium pH was obtained and then 20 µl of 1.0148 M HCl was added to each solution, and the equilibrium pH was remeasured when there was no further change in the measured pH with time. To obtain more equilibrium pH values, the addition of 20 µl of 1.0148 M HCl was repeated. The pH meter (HANNA Instruments, Woonsocket, RI) was calibrated at 25°C, and the equilibrium pH was measured utilizing a pH electrode (Sensorex, Garden Grove, CA) at 25°C while the solution was mixed.

RESULTS AND DISCUSSION

Comparison of Predicted Equilibrium pH with the Measured Equilibrium pH

Equilibrium pH values were obtained for each buffer pair, and in total, 70 equilibrium pH values were used in our analysis. The data utilizing the HEPES-MES buffer pair is shown graphically as a titration curve in Fig. 1. Figure 1
Table 1. Predicted pH vs. measured pH

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Factors Involved in the Prediction of the Equilibrium pH of a Multiple-Buffered Solution

A multiple-buffered aqueous solution, the addition of external protons results in a change in H⁺ partitioning among the various buffer pairs depending on their concentrations (activities) and their respective equilibrium constants. The need to predict final equilibrium pH values and to quantitate the magnitude of proton partitioning among various buffer constituents encompasses many fields including chemistry, pharmaceutical industry, physiology, and the clinical sphere. The predictive power of the Predictive Multiple Buffer Equation will therefore be of utility in these various areas that utilize equilibrium pH measurements.

Significant changes in the [H⁺] resulting from the addition of a proton load to an aqueous solution are prevented by the process of H⁺ buffering. Weak acid (release protons) and weak base (bind protons) buffer pairs, by mediating proton transfer reactions at a picosecond time scale, prevent large changes in [H⁺] from occurring. The ability of a particular buffer to ameliorate changes in the [H⁺] is determined by its concentration and its equilibrium constant Kᵢ, which is calculated based on the initial reactant concentrations expressed in molar concentrations and the apparent equilibrium constant Kᵢ of each buffer calculated according to Eq. 23. As demonstrated in Table 2, the Predictive Multiple Buffer Equation accurately predicted the measured equilibrium pH values. Not only is the Predictive Multiple Buffer Equation extremely accurate in predicting the equilibrium pH, we also show its utility in predicting the partitioning of H⁺ among the different buffer pairs at equilibrium (Table 2). In Table 2, the partitioning of H⁺ among the different buffer pairs at equilibrium is calculated according to Eqs. 17, 18, and 22, and the equilibrium [H⁺] is calculated as the difference between the initial [H⁺] and the sum of the concentrations of H⁺ being buffered by each of the individual buffer pairs (Eq. 4).

Factors Involved in the Prediction of the Equilibrium pH

In a multiple-buffered aqueous solution, the addition of external protons results in a change in H⁺ partitioning among the steady-state [H⁺] when the buffering of excess H⁺ by the various buffers is complete. Kᵢ, dissociation constant for water.

\[
[H⁺] \text{ refers to the initial H⁺ concentration (}[H⁺]\text{) immediately upon addition of HCl and before buffering of excess H⁺ by the various buffers. } \text{pHf refers to the steady-state [H⁺] when the buffering of excess H⁺ by the various buffers is complete. } K_i, \text{ dissociation constant for water.}
\]


deptics the titration plot of the predicted equilibrium pH vs. HCl (x) and measured equilibrium pH vs. HCl (O) using the HEPES-MES buffer pair. The results of all predicted and measured data points are shown in a master plot in Fig. 2. The linear least squares fit equation comparing all predicted to measured values had an \( r^2 \) value of 1, with a slope of 1, and an intercept of zero (Fig. 2). In Table 1, the Predictive Multiple Buffer Equation (Eq. 11) is used to determine the predicted equilibrium pH calculated based on the initial reactant concentrations expressed in molar concentrations and the apparent equilibrium constant \( K_i \) of each buffer calculated according to Eq. 23. As demonstrated in Fig. 1 and 2 and Table 1, the Predictive Multiple Buffer Equation accurately predicted the measured equilibrium pH values. Not only is the Predictive Multiple Buffer Equation extremely accurate in predicting the equilibrium pH, we also show its utility in predicting the partitioning of H⁺ among the different buffer pairs at equilibrium (Table 2). In Table 2, the partitioning of H⁺ among the different buffer pairs at equilibrium is calculated according to Eqs. 17, 18, and 22, and the equilibrium [H⁺] is calculated as the difference between the initial [H⁺] and the sum of the concentrations of H⁺ being buffered by each of the individual buffer pairs (Eq. 4).

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this regard, they do not per se differ from the Henderson-Hasselbalch equation. In a multiple-buffered solution at equilibrium, only one buffer pair is required to calculate the solution pH because of the isohydric principle, and therefore the more simple Constable equations are identical quantitatively in terms of their the isohydric principle, there is no need to utilize the equilibrium by 10.220.33.5 on April 30, 2017 http://ajprenal.physiology.org/ Downloaded from
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solution (8). Our present analysis indicates that the reported deviations in the Henderson-Hasselbalch equation and the Stewart strong ion formulation are simply a result of the inaccuracy in the equilibrium constants used in the calculations of equilibrium pH. Since both the Henderson-Hasselbalch equation and the Stewart strong ion formulation calculate the equilibrium pH based on the reactant molar concentrations (rather than ionic activities), the apparent equilibrium constants (rather than the thermodynamic equilibrium constants) of the various buffer pairs should therefore be used to determine the equilibrium pH. Consequently, previously reported deviations in the Henderson-Hasselbalch equation and the Stewart strong ion formulation are simply a result of the inaccuracies in the equilibrium constants used in the calculations of equilibrium pH.

Importantly, the Henderson-Hasselbalch equation and the Stewart strong ion formulation are not able to predict what the equilibrium pH will be following proton addition to a multiple-buffered aqueous solution. These formulas can be used to calculate the equilibrium pH only when the reactant concentrations at equilibrium are already known, and therefore they have no predictive capability. In predicting the equilibrium [H\(^+\)], we present a new quantitative approach to predicting the equilibrium [H\(^+\)] based on the preequilibrium concentrations of all the buffer pairs in a solution. This new mathematical model is the first quantitative approach that can predict the equilibrium [H\(^+\)] based on the partitioning of H\(^+\) buffering among the different buffer pairs in solution. Simply stated, the equilibrium [H\(^+\)] must be equal to the difference of the initial [H\(^+\)] and the sum of the concentrations of H\(^+\) being buffered by each of the individual buffer pairs:

\[
[H^+]_{\text{equilibrium}} = [H^+]_{\text{initial}} - x - y - z
\]

where \(x\), \(y\), and \(z\) represent the concentrations of H\(^+\) being buffered by each of the individual buffer pairs. In addition, based on the law of conservation of mass, for a given buffer pair

\[
[A^-]_{\text{equilibrium}} = [A^-]_{\text{initial}} - y
\]

\[
[HA^-]_{\text{equilibrium}} = [HA^-]_{\text{initial}} + y
\]

Our new mathematical model is also based on the isohydric principle (Eq. 11). According to the isohydric principle, the equilibrium [H\(^+\)] is equal to the product of the acid/base ratio (e.g., [HA]/[A\(^-\)]) of each buffer pair and its equilibrium constant. Since H\(_2\)O is the solvent for all buffers in solution, the water association/dissociation equilibrium also participates in proton buffering and must be considered as a separate buffer itself. However, in the case of H\(_2\)O, since the activity of H\(_2\)O is assumed to be approximately equal to 1 (7)

\[
[H^+]_{\text{equilibrium}} = K_{\text{w}}[OH^-]_{\text{equilibrium}}
\]

Based on all the above mathematical relationships, we derived a fourth-order polynomial equation which can be utilized to predict the equilibrium [H\(^+\)] in a multiple-buffered solution based on the preequilibrium reactant concentrations. The four possible roots of this fourth-order polynomial equation can be solved algebraically, or alternatively, the roots of this equation can be easily computed using user-friendly mathematical software such as wxMaxima and Mathematica. Although there are four possible roots to this polynomial equation, there is only one solution that will fulfill all the above mathematical relationships upon which the model is based on (Eq. 11)

<table>
<thead>
<tr>
<th>Experiment 5</th>
<th>[H(^+)], mol/l</th>
<th>H(^+) Buffered by OH(^-), mol/l</th>
<th>H(^+) Buffered by HEPES, mol/l</th>
<th>H(^+) Buffered by Acetate, mol/l</th>
<th>[H(^+)], mol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffers: OH(^-), HEPES, and acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0128E-03</td>
<td>1.1712E-06</td>
<td>1.0092E-03</td>
<td>2.3906E-06</td>
<td>9.5967E-09</td>
<td></td>
</tr>
<tr>
<td>1.0118E-03</td>
<td>3.7635E-07</td>
<td>1.0080E-03</td>
<td>3.3929E-06</td>
<td>1.8026E-08</td>
<td></td>
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<tr>
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<td>1.0053E-03</td>
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<tr>
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<td>1.0479E-07</td>
<td>1.0004E-03</td>
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<td>7.0076E-08</td>
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<tr>
<td>1.0079E-03</td>
<td>4.9140E-08</td>
<td>9.5056E-04</td>
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<tr>
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<tr>
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<td>8.2993E-04</td>
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<td>1.0087E-03</td>
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<td>3.0528E-05</td>
<td>9.7052E-04</td>
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<td>1.0123E-03</td>
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<td>1.1344E-05</td>
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<td>1.0263E-03</td>
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</table>

<table>
<thead>
<tr>
<th>Experiment 6</th>
<th>[H(^+)], mol/l</th>
<th>H(^+) Buffered by OH(^-), mol/l</th>
<th>H(^+) Buffered by MES, mol/l</th>
<th>H(^+) Buffered by Acetate, mol/l</th>
<th>[H(^+)], mol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffers: OH(^-), MES, and acetate</td>
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<td></td>
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<td></td>
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<td>2.4842E-07</td>
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<td>9.3046E-04</td>
<td>8.1114E-05</td>
<td>4.9220E-07</td>
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<tr>
<td>1.0113E-03</td>
<td>5.7439E-09</td>
<td>8.9136E-04</td>
<td>1.1882E-04</td>
<td>8.4218E-07</td>
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<tr>
<td>1.0107E-03</td>
<td>3.1992E-09</td>
<td>8.2188E-04</td>
<td>1.8736E-04</td>
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<tr>
<td>1.0102E-03</td>
<td>2.0088E-09</td>
<td>7.1803E-04</td>
<td>2.8964E-04</td>
<td>2.3513E-06</td>
<td></td>
</tr>
<tr>
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<td>1.2671E-09</td>
<td>5.7261E-04</td>
<td>4.3371E-04</td>
<td>3.8466E-06</td>
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<tr>
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<td>7.6079E-10</td>
<td>4.0500E-04</td>
<td>5.9938E-04</td>
<td>6.3655E-06</td>
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</tr>
<tr>
<td>1.0124E-03</td>
<td>4.4617E-10</td>
<td>2.6379E-04</td>
<td>7.3815E-04</td>
<td>1.0430E-05</td>
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<tr>
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<td>1.7084E-04</td>
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<tr>
<td>1.0211E-03</td>
<td>1.7300E-10</td>
<td>1.1355E-04</td>
<td>8.8000E-04</td>
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<td>1.0303E-03</td>
<td>1.2066E-10</td>
<td>8.1149E-05</td>
<td>9.0339E-04</td>
<td>4.5765E-05</td>
<td></td>
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</tbody>
</table>
\[ [H^+] = [H^+]_0 - y - z = \frac{K_w}{[OH^-]} - x \]

\[ K_w = K'_w([HA]_0 + y) = K'_b([HB]_0 + z) \]

Theoretically, this new quantitative approach can be expanded to account for a multiple buffered solution containing more than three buffers as needed.

The strong ion approach to acid-base analysis differs from our formulation in that the Stewart/Constable equations require the insertion of the reactant concentration at equilibrium and therefore are not predictive (1–3, 8, 12–14). Moreover, these equations are interpreted as demonstrating that the law of electroneutrality plays a pivotal role in defining the equilibrium [H⁺] of a solution. However, it has recently been shown that electroneutrality requirements (changes in strong ion difference) do not specify a given [H⁺] value (9). The fact that changes in strong ion difference do not specify a given [H⁺] can be demonstrated as shown in Table 3. Start with a 1-liter solution of 150 mM (0.15 M) NaCl in water with an initial strong ion difference \( \Delta = 0 \), [H⁺] = 1 × 10⁻³ M, [OH⁻] = 1 × 10⁻³ M, and [H⁺] − [OH⁻] = 0 (Table 3). If one were to add 100 mM (0.1 M) HCl, the strong ion difference would decrease to −100 mM (−0.1 M), and the [H⁺] − [OH⁻] difference would also increase to 100 mM (0.1 M). The excess H⁺ will then be consumed by OH⁻ to form H₂O. Since H⁺ and OH⁻ are consumed equally to form H₂O, the [H⁺] − [OH⁻] difference remains unchanged. The H⁺ and OH⁻ will continue to be consumed to form H₂O until the product of [H⁺] × [OH⁻] is equal to \( K_w \). Importantly, at the new strong ion difference of −0.1 M, electroneutrality is maintained (i.e. \([Na^+] + [H^+] - [Cl^-] - [OH^-] = 0 \)) regardless of the actual value of the [H⁺]. Therefore, the [H⁺] can be any value for a given change in strong ion difference, and the [H⁺] at equilibrium is only determined by the dissociation constant of water, \( K_w \). This holds true regardless of the absolute value of \( K_w \) (due to effects of ionic strength). Recently, we have derived the relationship between the equilibrium pH value and the concentrations of multiple buffer pairs in an aqueous solution that is not based on electroneutrality considerations (9). However, like the Henderson-Hasselbalch and Stewart/Constable equations, this equation requires that the reactant concentrations at equilibrium are known, and therefore this equation too is not predictive (9).

Previously, a predictive formula has been derived utilizing charge balance (macroscopic electroneutrality constraints), conservation of mass, and acid-base equilibria (11). Unlike the Stewart and Constable nonpredictive equilibrium-based equations, this equation is predictive because the total equilibrium concentrations of all weak acids and bases are assumed to be equal to the total preequilibrium concentrations of weak acids and bases according to the law of conservation of mass. In the derivation of this formula, the use of charge balance was mathematically convenient, however, as we have shown, is not mechanistically involved in predicting or determining the equilibrium pH value. Since the pH of a multiple-buffered aqueous solution is based mechanistically on the partitioning of excess H⁺ among the various buffers, we have derived a predictive formula based on partitioning of H⁺ buffering, conservation of mass, and acid-base equilibria. Moreover, the fact that our mathematical model can accurately predict the equilibrium pH based only on the partitioning of H⁺ buffering without requiring the incorporation of electroneutrality considerations provides definitive proof that electroneutrality plays no role in determining the pH of a solution.

### Table 3. Proof that a change in strong ion difference does not determine a specific [H⁺]

<table>
<thead>
<tr>
<th>Initial State</th>
<th>[Na⁺], mol/l</th>
<th>[Cl⁻], mol/l</th>
<th>SID, mol/l</th>
<th>[OH⁻], mol/l</th>
<th>[H⁺], mol/l</th>
<th>([Na^+] + [H^+] - [Cl^-] - [OH^-] )</th>
<th>([OH^-] \times [H^+] ), (mol/l)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add 0.1 mole HCl per liter of H₂O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preequilibrium</td>
<td>0.15</td>
<td>0.25</td>
<td>−0.1</td>
<td>1×10⁻⁷</td>
<td>0.0000001</td>
<td>0</td>
<td>1×10⁻¹⁴</td>
</tr>
<tr>
<td>Preequilibrium</td>
<td>0.15</td>
<td>0.25</td>
<td>−0.1</td>
<td>1×10⁻⁸</td>
<td>0.10000001</td>
<td>0</td>
<td>1×10⁻¹⁴</td>
</tr>
<tr>
<td>Preequilibrium</td>
<td>0.15</td>
<td>0.25</td>
<td>−0.1</td>
<td>1×10⁻⁹</td>
<td>0.10000001</td>
<td>0</td>
<td>1×10⁻¹⁴</td>
</tr>
<tr>
<td>Preequilibrium</td>
<td>0.15</td>
<td>0.25</td>
<td>−0.1</td>
<td>1×10⁻¹⁰</td>
<td>0.1000000001</td>
<td>0</td>
<td>1×10⁻¹⁴</td>
</tr>
<tr>
<td>Preequilibrium</td>
<td>0.15</td>
<td>0.25</td>
<td>−0.1</td>
<td>1×10⁻¹¹</td>
<td>0.1000000001</td>
<td>0</td>
<td>1×10⁻¹⁴</td>
</tr>
<tr>
<td>Preequilibrium</td>
<td>0.15</td>
<td>0.25</td>
<td>−0.1</td>
<td>1×10⁻¹²</td>
<td>0.10000000001</td>
<td>0</td>
<td>1×10⁻¹⁴</td>
</tr>
<tr>
<td>New equilibrium</td>
<td>0.15</td>
<td>0.25</td>
<td>−0.1</td>
<td>1×10⁻⁵</td>
<td>0.100000000001</td>
<td>0</td>
<td>1×10⁻⁵</td>
</tr>
</tbody>
</table>
predicted accurately. As demonstrated in Table 1 and Fig. 2, our results confirmed the accuracy of our new quantitative approach for predicting the final equilibrium pH. Indeed, linear regression analysis demonstrated that the predicted pH as calculated by our model is in excellent agreement with the measured pH: predicted pH = measured pH + 0.0038 ($r^2 = 1.0; 95\%$ CI slope = 1 to 1; $95\%$ CI intercept = $-0.0322$ to $0.0398$, where CI is the confidence interval).

### Physiological Relevance of the Predictive Multiple Buffer Equation

Our study has significant physiological and clinical relevance. First, there is much controversy as to the role of electroneutrality in determining the equilibrium pH. In this study, we have demonstrated experimentally for the first time that the equilibrium pH can be accurately predicted based solely on the partitioning of H$^+$ buffering among the buffer pairs in solution and that electroneutrality requirements do not play a role in defining the equilibrium pH of a solution. As shown in Table 2, by defining the partitioning of H$^+$ buffering among the different buffer pairs, the equilibrium [H$^+$] can therefore be predicted based on the difference between the initial [H$^+$] and the sum of the concentrations of H$^+$ being buffered by each of the individual buffer pairs without requiring calculations or considerations based on electroneutrality requirements. Second, our study also highlights the important fact that any mathematical model aimed at defining acid-base chemistry can only be predictive when it is based on pre-equilibrium reactant concentrations. In contrast to our mathematical model, previous approaches used in the analysis of acid-base chemistry (Henson-Hasselbalch equation and the Stewart strong ion formulations) can only describe (rather than predict) the equilibrium pH of a multiple-buffered solution. Importantly, these approaches calculate the equilibrium pH only when the reactant concentrations are already known. In this regard, it is simpler to directly measure the equilibrium pH rather than measuring the equilibrium reactant concentrations to calculate the equilibrium pH.

Our new mathematical model is the first reported quantitative approach that can predict the equilibrium [H$^+$] based on the partitioning of H$^+$ buffering among monoprotic buffers in solution. Since biological buffers (bicarbonate, phosphate, and albumin) are polyprotic in nature, a different and more complex model is necessary. Consequently, to the extent that these polyprotic biological buffers can be treated as monoprotic buffers in their derivations even though these biological buffers are truly polyprotic in nature. Consequently, to the extent that these polyprotic biological buffers can be accurately modeled as monoprotic buffers, our model can be used to predict and define the partitioning of H$^+$ among bicarbonate and phosphate/albumin buffers as well.

### Potential Sources of Error

In our calculation of the predicted equilibrium pH, the value of the thermodynamic equilibrium pK of each of the buffer pairs was based on reported values at 25°C. In refining the predictive accuracy of our model, the apparent equilibrium pK was utilized to calculate the final equilibrium pH since the reactant concentrations were expressed in molar concentrations (4). The apparent equilibrium pK was calculated based on the thermodynamic equilibrium pK and the predicted ionic strength of the solution at equilibrium according to Eq. 23 (4, 10). Consequently, errors in either the reported values and/or the correction for ionic strength would introduce discrepancies between predicted and measured values. An additional source of error is in the pH electrode measurement accuracy. However, despite these potential sources of error, the measured values were not significantly different from the values predicted using our formula, demonstrating that these potential sources of error were not biasing our results.

### Summary

We report the derivation of a new formula to predict the equilibrium pH based on the pre-equilibrium concentrations of the reactants in a multiple-buffered solution. Our new mathematical model indicates that the equilibrium [H$^+$] can be accurately predicted based solely on the partitioning of H$^+$ buffering among the buffer pairs in solution. Importantly, this model complements a previous analysis demonstrating that electroneutrality requirements do not play any role in defining the equilibrium [H$^+$] of a solution. To date, this is the first quantitative approach that can predict the equilibrium [H$^+$] based on the partitioning of H$^+$ buffering among the buffer pairs in solution.

### GRANTS

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### REFERENCES