Chapter 11 from *Stewart's Textbook of Acid-Base*

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[Chapter 11]

Role of Non-Volatile Weak Acids (Albumin, Phosphate and Citrate)

James Figge

11.1 The Stewart Model

In order to take the concepts developed by Stewart in chapters 1-9 further, let us review what the Stewart model tells us. First, the model characterizes all non-volatile weak acids in plasma (e.g., $H_2PO_4^-$, albumin, globulins, and the divalent citrate ion) collectively as a single species of weak acid, HA, which dissociates to H^+ and A^-. The dissociation reaction is governed by the apparent equilibrium dissociation constant, K_A:

$$K_A = (\alpha H^+) \times [A^-] / [HA] \qquad (11.1.1)$$

where (αH^+) is the hydrogen ion activity. The concentrations of the acid, [HA], and conjugate base, $[A^-]$, are in mol / L. Conservation of mass is required per the equation:

$$[A_{TOT}] = [HA] + [A^-] \qquad (11.1.2)$$

where $[A_{TOT}]$ is given in mol / L. Hence,

$$[HA] = [A_{TOT}] - [A^-]$$

Given that $pH = - \log_{10}(\alpha H^+)$ and $pK_A = - \log_{10}(K_A)$, equations 1 and 2 can be combined and algebraically rearranged to give:

$$pH = pK_A + \log_{10}([A^-] / \{ [A_{TOT}] - [A^-] \}) \qquad (11.1.3)$$

The model postulates that the negative charge contributed by the conjugate base, $- [A^-]$, is equal to the net negative charge (in Eq / L) contributed by plasma proteins and other non-volatile weak acids and their conjugate bases

(e.g., $H_2PO_4^-$, HPO_4^{2-}, di- and trivalent citrate species). Equation 3 can be rearranged to solve for $[A^-]$ when $[A_{TOT}]$, pK_A, and pH are known:

$$[A^-] = [A_{TOT}] / (1 + 10 \text{ ^ } \{pK_A - pH\}) \quad (11.1.4)$$

The expression employed by Stewart for the carbon dioxide - bicarbonate equilibrium is mathematically equivalent to the classic Henderson-Hasselbalch equation:

$$pH = 6.1 + \log_{10}([HCO_3^-] / \{0.0307 \times PCO_2\}) \quad (11.1.5)$$

where $[HCO_3^-]$ is in mmol / L and PCO_2 is in mm Hg. A modern value for the constant, K_C, used by Stewart, can be calculated as follows:

$$K_C = (10^{-6.1} \text{ mol / L}) \times (0.0307 \text{ mmol / L / mmHg}) \times (1 \text{ mol / 1000 mmol})$$
$$= 2.44 \times 10^{-11} \text{ (mol / L)}^2 \text{ / mmHg} \quad (11.1.6)$$

By rearranging equation 11.1.5 and substituting in K_C, one obtains:

$$[HCO_3^-] = K_C \times PCO_2 / (\alpha H^+) \quad (11.1.7)$$

where $[HCO_3^-]$ is in mol / L.

In accounting for electrical neutrality, the Stewart model includes completely dissociated non-reacting ionic species at physiologic pH (strong ions) as well as weak acids and their conjugate bases harboring a charge. To account for the net contribution of strong ions, Stewart introduced the quantity "strong ion difference" (SID). This quantity can be defined as:

$$[SID] = [Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}] + [XC^+] - [Cl^-] - [Lactate^-] - [XA^-] \quad (11.1.8)$$

where XC^+ and XA^- are miscellaneous (unmeasured) strong cations and strong anions, respectively, and all quantities are in Eq / L. Note that if $[Lactate^-]$ is not specifically measured, then its value is included in $[XA^-]$. The SID is a convenient mathematical shortcut to simplify the calculation of electrical neutrality with respect to the contribution of relevant strong ions. The requirement for electrical neutrality can be written as:

$$[SID] + [H^+] - [HCO_3^-] - [CO_3^{2-}] - [OH^-] - [A^-] = 0 \quad (11.1.9)$$

For practical purposes, $[H^+]$, $[CO_3^{2-}]$ and $[OH^-]$ are negligible within the physiologic pH range and can be omitted from equation 11.1.9 without introducing a significant error.

11.2 Quantitative Physicochemical Models of Plasma Featuring Detailed Analyses of Albumin

Figge, Rossing and Fencl [1] developed an extension of Stewart's quantitative physicochemical model of human acid-base physiology. Unlike the original Stewart model, the Figge-Rossing-Fencl model treats human serum albumin as a polyprotic macromolecule with multiple apparent equilibrium dissociation constants corresponding to different classes of amino acid side chains. The Figge-Rossing-Fencl model also employs a robust mathematical treatment of the phosphoric acid - phosphate system (i.e., H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}). The simultaneous equilibria as treated in the Figge-Rossing-Fencl model include statements of dissociation for various non-volatile weak acids (e.g., ionizable amino acid side chains, the phosphoric acid - phosphate system) as well as the carbon dioxide – carbonic acid - bicarbonate - carbonate system.

As for the Stewart model, the Figge-Rossing-Fencl model incorporates three fundamental physicochemical principles as they apply to a single body fluid compartment (such as arterial blood plasma) under steady-state conditions:

- conservation of mass;
- electrical neutrality; and
- simultaneous satisfaction of multiple dissociation equilibria.

The Figge-Rossing-Fencl model was developed using data from electrolyte solutions resembling human serum that contained albumin as the sole protein moiety. This is not only successful in calculating the pH of albumin-containing electrolyte solutions, but it also accurately calculates the pH of filtrands of serum which contain both albumin and globulin. This finding demonstrates that globulins contribute only negligibly to acid-base balance in humans within the pH range of biologic significance. Possible reasons for this finding will be discussed below.

The quantitative physicochemical model was further refined by Figge, Mydosh and Fencl [2] to incorporate apparent pK_A values for albumin histidine residues as determined by NMR-spectroscopy [3] (temperature-corrected from 25 to 37 ^oC using data from a model compound). This is of particular interest because histidine residues can titrate within or near the physiologic pH range. The Figge-Mydosh-Fencl model recognizes the fact that apparent pK_A values of individual histidine residues vary as a function of the microenvironment within various regions of the protein.

Although the Figge-Mydosh-Fencl model is successful in many respects, it does not account for the presence of all 59 lysine residues in human serum albumin. Figge, Mydosh and Fencl hypothesized that several lysine residues are buried within the interior of albumin and are not accessible to the solvent. Consequently, the buried lysine residues are not included in acid-base balance

calculations in the Figge-Mydosh-Fencl model. However, an alternative hypothesis is that several lysine residues exist in albumin with unusually low pK_A values. This hypothesis is plausible because low-titrating lysine residues have been documented in other proteins [4]. Furthermore, the existence of low-titrating lysine residues in human serum albumin is supported by fluorescence emission spectroscopic data [5] interpreted with reference to the x-ray crystallographic structure of albumin [6-7].

Consequently, a quantitative physicochemical model of human acid-base balance in blood plasma has been developed to demonstrate that low-titrating lysine residues can be successfully incorporated into the model calculations. The Figge-Fencl Quantitative Physicochemical Model of Human Acid-Base Physiology [8] is completely described online at www.Figge-Fencl.org. The Figge-Fencl model explicitly accounts for the contribution of all 59 lysine residues within human serum albumin. The model features a small number of lysine residues with unusually low pK_A values (Appendix 1). The model also incorporates all of the advanced features of the Figge-Rossing-Fencl and Figge-Mydosh-Fencl models as described above. In addition, the temperature correction from 25 to 37 ^oC for pK_A values of albumin histidine residues is calculated using the van't Hoff equation (see Chapter 12 for details).

Human serum albumin undergoes several structural transitions as a function of pH. The N-B (neutral-to-base) transition [3, 5] occurs between pH 6 to 9 and is important because this interval includes the physiologic pH range. The Figge-Fencl Quantitative Physicochemical Model [8] incorporates an empiric function that simulates the N-B transition by downshifting the pK_A values of five histidine residues as the pH increases from 6 to 9.

To test the validity of a quantitative model, one can verify whether or not the model accurately predicts independent experimental data. The albumin titration curve calculated directly from the Figge-Fencl model gives the predicted number of charges per albumin molecule as a function of pH at 37 ^oC. The predicted titration curve over the pH range of 5 to 9 is given in Figure 11.1 (smooth curve). This is plotted against independent experimental data points of Fogh-Andersen, Bjerrum and Siggaard-Andersen [9]. These data points (taken from Figure 4 in reference [9]) give the intrinsic charge carried by a molecule of human serum albumin at 37 ^oC for a given pH (diamonds). As demonstrated in Figure 1, the Figge-Fencl model predictions agree very closely with the independent experimental data over the pH range of 5.05 to 8.75. In contrast, the Figge-Mydosh-Fencl model is valid only within a narrower pH range of 6.9 to 7.9 [8]. This analysis demonstrates that the Figge-Fencl model, which postulates the existence of low-titrating lysine residues, is a more accurate representation of the true acid-base titrating behavior of albumin over the pH range of 5 to 9. Therefore, the calculations from the Figge-Fencl model [8] will be the basis for the application program discussed later in this chapter.

11.3 Charge Contributed by Albumin and Molar Buffer Capacity of Albumin over the pH Interval of 6.9 to 7.9.

The Figge-Fencl model [8] predicts that the titration curve of human serum albumin (intrinsic negative charge displayed by albumin, expressed as mEq per g of albumin, versus pH) is approximately linear over the pH interval of 6.9 to 7.9. The approximate negative charge contributed by albumin in human plasma over this pH range as predicted by the Figge-Fencl model is given by:

$$[Z_{ALBUMIN}] = - 10 \times [Albumin] \times (0.1204 \times pH - 0.625) \qquad (11.3.1)$$

where $[Z_{ALBUMIN}]$ is in mEq / L and [Albumin] is in g / dL. Therefore, at pH 7.40, the charge contributed by 4.40 g / dL of albumin is approximately -11.7 mEq / L. The molar buffer capacity of albumin within the pH interval of 6.9 to 7.9 can be calculated from the slope in this equation (0.1204 mEq / g / pH):

$$(0.1204 \text{ mEq / g / pH}) \times (66500 \text{ g / mol}) \times (1 \text{ Eq / 1000 mEq}) = 8.0 \text{ Eq / mol / pH} \qquad (11.3.2)$$

which is identical to the value given by Siggaard-Andersen and Fogh-Andersen [10]. The molar buffer capacity is used in the derivation of the van Slyke equation for plasma and is discussed below.

11.4 Plasma Globulins

A detailed model for the contribution of plasma globulins is difficult, if not impossible, to develop due to the marked heterogeneity of plasma globulin species. As previously noted [1], the overall contribution of plasma globulins to human acid-base physiology is small, most likely due to the wide distribution of isoelectric points for individual globulin molecules. Based on liquid phase preparative isoelectric focusing of native human immunoglobulin molecules [11], the distribution of isoelectric points for human IgG molecules is 4.35 to 9.95, with a dominant peak between pH 7 and 9.95, centered at pH 8.2. Hence, in the physiologic pH range, a large fraction of IgG molecules will bear a positive charge since a significant proportion of IgG molecules have isoelectric points > 7.8. The distribution of isoelectric points for IgA molecules [11] is between 4 and 7.1, with a peak between pH 4.7 and 5.9. IgM molecules [11] demonstrate a distribution of isoelectric points ranging between 4 and 9.1, with a peak between pH 5.5 and 6.7. The alpha-globulin and beta-globulin fractions typically have isoelectric points < 7.0 and will be negatively charged in the physiologic pH range. Hence, at physiologic pH values, the positive charges contributed by plasma IgG molecules are predicted to at least partially counterbalance the negative charges contributed by the alpha- and beta-globulin fractions as well as IgA, the majority of IgM molecules and the remaining IgG molecules. The actual

net charge contributed by globulins within the physiologic pH range is not known with certainty and will require additional experimental measurement. The net charge contributed by total plasma proteins can, in theory, be derived by adding the intrinsic charge of albumin to the net charge contributed by globulins. Rough estimates of this value have been developed by van Leeuwen [12].

11.5 Charge Contributed by the Phosphoric Acid – Phosphate System

Following the work of Sendroy and Hastings [13] the concentration of each phosphorus-containing species is given by the following set of equations:

$$[H_3PO_4] = [P_{i,TOT}] \times (\alpha H^+)^3 / Y \quad (11.5.1)$$

$$[H_2PO_4^-] = [P_{i,TOT}] \times (\alpha H^+)^2 \times K_1' / Y \quad (11.5.2)$$

$$[HPO_4^{2-}] = [P_{i,TOT}] \times (\alpha H^+) \times K_1' \times K_2' / Y \quad (11.5.3)$$

$$[PO_4^{3-}] = [P_{i,TOT}] \times K_1' \times K_2' \times K_3' / Y \quad (11.5.4)$$

where:

$$Y = (\alpha H^+)^3 + \{ (\alpha H^+)^2 \times K_1' \} + \{ (\alpha H^+) \times K_1' \times K_2' \} + \{ K_1' \times K_2' \times K_3' \} \quad (11.5.5)$$

and $[P_{i,TOT}]$ is in mmol / L. Constants K_1', K_2' and K_3' are the apparent equilibrium dissociation constants for phosphoric acid. For plasma [13], $K_1' = 1.22 \times 10^{-2}$ (or $pK_1' = 1.915$); $K_2' = 2.19 \times 10^{-7}$ (or $pK_2' = 6.66$); and $K_3' = 1.66 \times 10^{-12}$ (or $pK_3' = 11.78$).

The net charge contributed by all of these species in mEq / L is given by:

$$[Z_{Pi}] = - ([H_2PO_4^-] + 2 \times [HPO_4^{2-}] + 3 \times [PO_4^{3-}]) \quad (11.5.6)$$

Define Z_P as:

$$Z_P = (\{ (\alpha H^+)^2 \times K_1' \} + \{ 2 \times (\alpha H^+) \times K_1' \times K_2' \} + \{ 3 \times K_1' \times K_2' \times K_3' \}) / Y \quad (11.5.7)$$

Then, it follows that:

$$[Z_{Pi}] = - [P_{i,TOT}] \times Z_P \quad (11.5.8)$$

At pH 7.40, $Z_P = 1.85$ mEq / mmol. Also at pH 7.40, the slope of Z_P versus pH, dZ_P / dpH, is 0.30 mEq / mmol / pH. Given a value for $[P_{i,TOT}]$ of 1.15 mmol / L, corresponding to 3.6 mg / dL of phosphorus, and a pH of 7.40, then $[Z_{Pi}] = -2.1$ mEq / L. About 99.99% of the charge is contributed by $H_2PO_4^-$ and HPO_4^{2-}.

11.6 Charge Contributed by the Citric Acid – Citrate System

Citric acid is a tri-carboxylic acid with approximate apparent equilibrium dissociation constants of 1.05×10^{-3} (or $pK_1' = 2.98$); 4.27×10^{-5} (or $pK_2' = 4.37$); and 1.62×10^{-6} (or $pK_3' = 5.79$) at 37 ^{o}C and ionic strength of 0.16 M (adjustments to the pK' values to account for the effect of ionic strength were calculated using the Debye-Hückel theory). The analytical approach for citrate mirrors that of phosphate:

$$[Z_{CITRATE}] = - [Citrate_{TOT}] \times Z_C \qquad (11.6.1)$$

At pH 7.40, $Z_C = 2.98$ mEq / mmol. Also at pH 7.40, the slope of Z_C versus pH is 0.054 mEq / mmol / pH, indicating that citrate is a poor plasma buffer in the physiologic pH range. The default value for plasma citrate concentration, $[Citrate_{TOT}]$, in the fasting state is 0.135 mmol / L, corresponding to 2.6 mg / dL [14]. Hence, at pH 7.40, $[Z_{CITRATE}] = -0.40$ mEq / L. About 99.999% of the charge is contributed by the divalent and trivalent citrate species.

11.7 Base Excess and Derivation of the van Slyke Equation for Plasma

Fencl, et al. [15], as well as Wooten [16], demonstrated that the Figge-Mydosh-Fencl model can be used to derive the classic van Slyke equation for plasma [10], thereby providing a theoretical link to traditional acid-base formulations as advocated by Siggaard-Andersen [10] and others (see chapter 14). The van Slyke equation is valid in situations where the concentrations of non-bicarbonate (i.e., non-volatile) buffers in plasma (albumin, globulin, phosphorus-containing species, and citrate species) remain constant. The equation can be used in combination with the Henderson-Hasselbalch equation to describe a change in state of a system such as arterial blood plasma. The van Slyke equation is:

$$BE = -\Delta ctH^+ = ([HCO_3^-] - [HCO_3^-]^\circ) + \beta_{pl} \times (pH - pH^\circ)$$

$$= \Delta[HCO_3^-] + \beta_{pl} \times \Delta pH \qquad (11.7.1)$$

where BE is the Base Excess; ctH^+ is the concentration of titratable hydrogen ion; $pH^\circ = 7.40$ (the reference pH value at $T^\circ = 37$ ^{o}C); and $[HCO_3^-]^\circ = 24.5$ mmol / L (the reference bicarbonate concentration at $T^\circ = 37$ ^{o}C, $PCO_2^\circ = 40$ mmHg (5.33 kPa)). Base Excess is defined by Siggaard-Andersen as the negative value of the concentration of titratable hydrogen ion in blood or plasma. Deviations from standard reference values are denoted as $\Delta[HCO_3^-] = ([HCO_3^-] - [HCO_3^-]^\circ)$, and $\Delta pH = (pH - pH^\circ)$. The parameter, β_{pl} , is the buffering capacity of non-bicarbonate buffers in plasma. This parameter is a constant provided that the concentrations of non-volatile buffering species remain constant. The parameter, β_{pl} , can be calculated for specific concentrations of these plasma buffers, as shown in the following example.

For [Albumin] of 4.4 g / dL, the buffer capacity of albumin is: 5.30 mEq / L / pH. The apparent specific buffer capacity of plasma globulins [10] is 0.075 mol / kg / pH. Therefore, for [Globulin] of 2.8 g / dL, the buffer capacity is 2.10 mEq / L / pH. Although globulins contribute only negligibly to electrical charge balance at physiologic pH values, it is expected that they have a significant buffering capacity as noted. For phosphorus-containing species, $[P_{i,TOT}]$ of 1.15 mmol / L, the buffer capacity at pH 7.40 is given by

$$0.30 \text{ mEq / mmol / pH x } 1.15 \text{ mmol / L} = 0.35 \text{ mEq / L / pH} \qquad (11.7.2)$$

For $[Citrate_{TOT}]$ of 0.135 mmol / L, the buffer capacity is negligible at pH 7.40.

Adding these together gives:

$$\beta_{pl} = 5.30 + 2.10 + 0.35 = 7.75 \text{ mEq / L / pH} \qquad (11.7.3)$$

very close to the default value of 7.7 given by Siggaard-Andersen and Fogh-Andersen [10].

By rearranging terms in the van Slyke equation, one obtains,

$$\Delta[HCO_3^-] = BE - \beta_{pl} \times \Delta pH \qquad (11.7.4)$$

By taking the first derivative of appropriate terms in the Figge-Fencl model, provided that the concentrations of non-volatile buffering species remain constant, one can derive the following equation that has the same form as the van Slyke equation [15, 16]:

$$\Delta[HCO_3^-] = \Delta[SID] - \beta_{pl} \times \Delta pH \qquad (11.7.5)$$

where $\Delta[SID] = ([SID] – [SID]°)$, and [SID]° is the reference value for [SID] at T° = 37 ^oC.

This result leads directly to the important conclusion that BE = Δ[SID], if and only if, the concentrations of non-bicarbonate (non-volatile) buffers remain constant. This is a critical result because it provides a unifying theoretical framework between Stewart's physicochemical formulation, including the concept of [SID], and the classical (Base Excess) theory of acid-base. This relationship was demonstrated mathematically by Schlichtig [17] and experimentally by Kellum et al. [18] who compared [SID] and BE across a vascular bed where $[A_{TOT}]$ was invariant.

The van Slyke equation for whole blood additionally accounts for buffering by hemoglobin. Wooten has published a sophisticated multi-compartment model that applies to human whole blood [19]. This approach brings the SID methodology to the same quantitative level as the Base Excess method.

11.8 Acid-Base Disturbances are Caused by Alterations in Independent Variables

Significant departures of independent variables from their standard physiologic reference values will result in acid-base disturbances. The Figge-Fencl model can be used to predict the magnitude of acid-base disturbances that will result from deviations in the independent variables from their physiologic reference values in plasma-like solutions containing albumin. This approach is not yet ready for clinical applications due to the fact that the exact contribution of plasma globulins has not been incorporated into the model. However, the model can serve to illustrate basic concepts underlying clinical acid-base disturbances. The current model can solve the following function (see also Appendix):

$$pH = f_{pH} \{ [SID], PCO_2, [P_{i,TOT}], [Albumin], [Citrate_{TOT}] \} \qquad (11.8.1)$$

While most metabolic acid-base disturbances result from significant deviations in SID from its standard physiologic reference value, alterations in $[P_{i,TOT}]$ and [Albumin] also produce metabolic acid-base disturbances, whereas alterations in PCO_2 produce respiratory disturbances. In critically ill patients, it is not uncommon to have disturbances in all four independent variables ([SID], [Albumin], $[P_{i,TOT}]$ and PCO_2). Citrate is usually present in concentrations near 0.135 mmol / L [14], so its effect on overall acid-base balance is very small.

Figures 2 through 4 show the graphic results from computer simulations in which the Figge-Fencl model is used to demonstrate the effect of varying [SID], PCO_2, and [Albumin] in plasma-like solutions containing albumin as the sole protein moiety. The simulation in Figure 2 is run using [Albumin] = 4.4 g / dL, whereas in Figure 3 the value of [Albumin] = 2.2 g / dL. In both figures the [SID] ranges from 20 to 50, and results are plotted with three different values of PCO_2 (20, 40, and 60 mm Hg). The simulation depicted in Figure 4 resembles a classic "Davenport diagram" [20] and demonstrates the covariation of pH and $[HCO_3^-]$ as the PCO_2 is titrated from 15 to 100 mm Hg at two values of [SID] (38.9 and 28.9 mEq / L). The curve for [SID] = 38.9 mEq / L resembles the buffer curve of normal separated human plasma [20]. The situation with [SID] = 28.9 resembles separated plasma with a simulated metabolic acidosis. The base excess, BE = Δ[SID] = 28.9 – 38.9 = -10 (i.e., there is a base deficit of 10 mmol / L per Davenport's nomenclature). The relationship BE = Δ[SID] holds in this example because the concentrations of albumin, $P_{i,TOT}$ and $Citrate_{TOT}$ are all held constant. Figure 4 is an important result because it demonstrates that the Stewart approach can actually be used to derive the classic bicarbonate-

centered approach to acid-base taken by Davenport [20], and it graphically demonstrates that BE = Δ[SID], again when the concentrations of all non-volatile buffers are held constant. These figures demonstrate the complex interplay between the independent variables and show the power of the computer model. An application program written in Visual Basic is available online at http://www.Figge-Fencl.org/ and can be downloaded free of charge for educational and academic use only. The program will solve the function, pH = f_{pH} { [SID], PCO_2, $[P_{i,TOT}]$, [Albumin], $[Citrate_{TOT}]$ }, for any legitimate combination of independent variables and can be used to develop simulations such as those demonstrated in Figures 2 through 4. As noted, this program should not be used for clinical applications.

11.9 Calculation of K_A and $[A_{TOT}]$

Stämpfli and Constable [21] have successfully determined the value of K_A and $[A_{TOT}]$ for a number of species including human. Their approach extends the original work of Stewart and is completely consistent with the Figge-Fencl model but also incorporates the effect of plasma globulins as well as albumin, phosphate and citrate. Further details follow in Chapter 12.

11.10 Summary

1. A series of advances in the original Stewart model have come about by careful examination of the non-volatile weak acids. These models treat human serum albumin as a polyprotic macromolecule with multiple apparent equilibrium dissociation constants corresponding to different classes of amino acid side chains; and employ a robust mathematical treatment of the phosphoric acid - phosphate system.

2. Although the net charge contributed by globulins within the physiologic pH range is not known with certainty, available evidence suggests that globulins have a negligible effect on acid-base balance.

3. The Figge-Mydosh-Fencl model can be used to derive the classic van Slyke equation for plasma and it can be shown that, when A_{TOT} is held constant, BE = Δ[SID].

Appendix: Formal Mathematical Representation of the Figge-Fencl Model

The following is the Formal Mathematical Representation [8] of the Figge-Fencl Quantitative Physicochemical Model of Human Acid-Base Physiology for Plasma-Like Solutions.

$[SID] + 1000 \times ((\alpha H^+) - K_W / (\alpha H^+) - K_{C1} \times PCO_2 / (\alpha H^+) - K_{C1} \times K_{C2} \times PCO_2 / (\alpha H^+)^2) - [P_{i,TOT}] \times Z_P - [Citrate_{TOT}] \times Z_C$

+ { -1 / (1 + 10 ^ - (pH - 8.5))

- 98 / (1 + 10 ^ - (pH - 4.0))

- 18 / (1 + 10 ^ - (pH - 11.7))

+ 24 / (1 + 10 ^ + (pH - 12.5))

+ 2 / (1 + 10 ^ + (pH - 5.80))

+ 2 / (1 + 10 ^ + (pH - 6.00))

+ 1 / (1 + 10 ^ + (pH - 7.60))

+ 2 / (1 + 10 ^ + (pH - 7.80))

+ 2 / (1 + 10 ^ + (pH – 8.00))

+ 50 / (1 + 10 ^ + (pH - 10.3))

+ 1 / (1 + 10 ^ + (pH - 7.19 + NB))

+ 1 / (1 + 10 ^ + (pH - 7.29 + NB))

+ 1 / (1 + 10 ^ + (pH - 7.17 + NB))

+ 1 / (1 + 10 ^ + (pH - 7.56 + NB))

+ 1 / (1 + 10 ^ + (pH - 7.08 + NB))

+ 1 / (1 + 10 ^ + (pH - 7.38))

+ 1 / (1 + 10 ^ + (pH - 6.82))

+ 1 / (1 + 10 ^ + (pH - 6.43))

+ 1 / (1 + 10 ^ + (pH - 4.92))

+ 1 / (1 + 10 ^ + (pH - 5.83))

+ 1 / (1 + 10 ^ + (pH - 6.24))

+ 1 / (1 + 10 ^ + (pH - 6.80))

+ 1 / (1 + 10 ^ + (pH - 5.89))

+ 1 / (1 + 10 ^ + (pH - 5.20))

+ 1 / (1 + 10 ^ + (pH - 6.80))

+ 1 / (1 + 10 ^ + (pH - 5.50))

+ 1 / (1 + 10 ^ + (pH - 8.0))

- 1 / (1 + 10 ^ - (pH - 3.1)) } x 1000 x 10 x [Albumin] / 66500 = 0

Where:

$(\alpha H^+) = 10^{-pH}$; (αH^+) is the hydrogen ion activity, also used as an approximation of hydrogen ion concentration, $[H^+]$;

$Z_P = (K_1 \times (\alpha H^+)^2 + 2 \times K_1 \times K_2 \times (\alpha H^+) + 3 \times K_1 \times K_2 \times K_3) / ((\alpha H^+)^3 + K_1 \times (\alpha H^+)^2 + K_1 \times K_2 \times (\alpha H^+) + K_1 \times K_2 \times K_3)$;

$Z_C = (C_1 \times (\alpha H^+)^2 + 2 \times C_1 \times C_2 \times (\alpha H^+) + 3 \times C_1 \times C_2 \times C_3) / ((\alpha H^+)^3 + C_1 \times (\alpha H^+)^2 + C_1 \times C_2 \times (\alpha H^+) + C_1 \times C_2 \times C_3)$;

NB = 0.4 x (1 - 1 / (1 + 10 ^ (pH - 6.9)));

$[Citrate_{TOT}]$ is given in mmol / L;

Strong Ion Difference, [SID], is given in mEq / L;

PCO_2 is given in mmHg;

Total concentration of inorganic phosphorus-containing species, $[P_{i,TOT}]$, is given in mmol / L;

[Albumin] is given in g / dL;

$K_W = 4.4 \times 10^{-14}$ (Eq / L)^2;

$K_{C1} = 2.44 \times 10^{-11}$ (Eq / L)^2 / mmHg;

$K_{C2} = 1.1 \times 10^{-10}$ (Eq / L);

$K_1 = 1.22 \times 10^{-2}$ (mol / L);

$K_2 = 2.19 \times 10^{-7}$ (mol / L);

$K_3 = 1.66 \times 10^{-12}$ (mol / L);

$C_1 = 1.05 \times 10^{-3}$ (mol / L);

$C_2 = 4.27 \times 10^{-5}$ (mol / L);

$C_3 = 1.62 \times 10^{-6}$ (mol / L);

66500 g / mol is the molecular weight of albumin.

The above expression defines a function, f_{pH}, which can be used to calculate the pH of plasma-like solutions containing albumin for any valid set of values for [SID], PCO_2, $[P_{i,TOT}]$, [Albumin], $[Citrate_{TOT}]$:

$$pH = f_{pH} \{ [SID], PCO_2, [P_{i,TOT}], [Albumin], [Citrate_{TOT}] \}.$$

The function is too complex to be solved by hand and must be solved via an iterative approach on a computer. An application program is available for this purpose [8].

The model includes the following features for human serum albumin:

Amino Acid	Number of Side Chains	Microenvironmental pK_A
Cys	1	8.5
Glu and Asp	98	4.0
Tyr	18	11.7
Arg	24	12.5
Lys	2	5.80
	2	6.00
	1	7.60
	2	7.80
	2	8.00
	50	10.3
His	1	7.19 *
	1	7.29 *
	1	7.17 *
	1	7.56 *
	1	7.08 *
	1	7.38
	1	6.82
	1	6.43
	1	4.92
	1	5.83
	1	6.24
	1	6.80
	1	5.89
	1	5.20
	1	6.80
	1	5.50
Amino terminus	1	8.0
Carboxyl terminus	1	3.1

* Note that the pK_A's of the first five His residues will each downshift by 0.4 pH units due to the structural rearrangement associated with the Neutral-to-Base (N-B) transition.

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