

ON THE MEASUREMENT OF BUFFER VALUES AND ON THE RELATIONSHIP OF BUFFER VALUE TO THE DISSOCIATION CONSTANT OF THE BUFFER AND THE CONCENTRATION AND REACTION OF THE BUFFER SOLUTION.*

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The Nature and Mode of Action of Buffers.

In his chapter on buffer action Clark (1920) has traced the development of the knowledge of buffers,¹ which we owe mainly

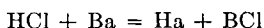
* The present paper constitutes the theoretical basis for experimental work, the results of which will follow.

¹ Clark (1920), p. 30.

to the work of L. J. Henderson (1908), and of Sørensen (1912), and has summarized the resultant conception in the statement: "By buffer action we mean the ability of a solution to resist change in pH through the addition or loss of alkali or acid." Reduced to the form of a definition this conception may be expressed as follows: *Buffers are substances which by their presence in solution increase the amount of acid or alkali that must be added to cause unit change in pH.* As will appear later, change in the logarithmic pH unit affords a more convenient measure of buffer effect than change in $[H^+]$.

The most efficient buffers, at reactions within the usual range of biological significance, are mixtures of weak acids or weak bases and their salts. Their buffer effect is due to the relatively slight extent to which they undergo electrolytic dissociation, as compared with the almost completely dissociated strong acids and bases.

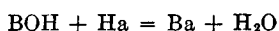
If we add a strong acid, HCl for example, to a mixture of the weak buffer acid, Ha, and its alkali salt, Ba, reaction occurs with approximate completeness from left to right according to the equation



(To represent the weak buffer acid the formula Ha is used to distinguish it from HA, which will be used to indicate a strong, approximately entirely dissociated acid.) In this reaction the strong acid combines with an equivalent of buffer salt and sets free an equivalent of weak buffer acid, Ha. The latter, instead of dissociating like HCl almost entirely into hydriions and anions, dissociates only slightly. Hence the increase in hydriion concentration, and the change in pH, is only a fraction of that which would have been caused by adding the same amount of HCl to an unbuffered solution.

The amount of change that does occur depends on the extent to which the buffer acid set free dissociates according to the equation $Ha = H^+ + a'$. If 1 per cent of Ha dissociates into H^+ and a' , the H^+ increase will be approximately 0.01 of that which would have been caused by adding the HCl to an unbuffered solution.

Similarly, if we add a strong base, BOH, such as KOH or NaOH, to the buffer solution, an equivalent of buffer acid in the Ba, Ha mixture is neutralized according to the equation



The decrease in H^+ concentration due to neutralization of a given amount of Ha is again dependent on the extent to which the Ha had been dissociated, since only the H^+ ions owing their presence to that dissociation have disappeared.

It is evident from the above that the Ba of the buffer mixture reacts when acid is added, and the Ha when alkali is added, and therefore that both Ba and Ha are necessary if the buffer mixture is to offer resistance to reaction change by addition of either acid or alkali. As will be seen later, if ability to minimize the proportion by which any given H^+ concentration is changed is taken as a measure of buffer action, a buffer mixture has its maximum efficiency when $Ha = Ba$, half the buffer being free, half in the form of its salt.

When the buffer mixture is composed of a weak base, bOH , and its salt, bA , it can be shown similarly that the amount of change in H^+ or OH' concentration caused by addition of acid or alkali depends on the extent to which bOH dissociates into b^+ and OH' ions.

Since weak acids and bases obey in their dissociation the simple law of mass action, and since their buffer action is dependent on the extent of their dissociation, it follows that the quantitative relationships governing buffer action are capable of formulation from the mass law.

By means of such formulation, L. J. Henderson (1908) has shown that buffer acids most efficient in maintaining a neutral reaction of $[H^+] = [OH'] = 10^{-7}$ are such as have dissociation constants most nearly equal to 10^{-7} . Clark (1920) has pointed out² "that it is only within a certain zone of $\log \frac{1}{[H^+]}$ that a mixture of an acid with its salt produces a stabilized hydrogen ion concentration or pH." The writer has recently indicated (1921, *a*) a mode for the mathematical proof that in general, if buffer efficiency be considered as ability to minimize change in pH, that is, proportional change in $[H^+]$, any buffer salt of a weak acid is most efficient when $[Ha]$ and $[Ba]$ are equal, under which conditions $pH = pK'_a$.³

² Clark (1920), p. 19.

³ pK'_a is an expression introduced by Hasselbalch (1917) to indicate the negative logarithm of K'_a , the value of K'_a being $\frac{K_a}{\gamma}$ where K_a is the dis-

Unit for Measurement of Buffer Values.

Thus far, however, there has appeared in the literature no satisfactory mode of expressing, over the zone of pH at which buffers act, the quantitative relationships of buffer effect to the dissociation constant of the buffer and the reaction: in fact there has been no unit for the numerical expression of buffer effect. It is the purpose of the present paper by means of such a unit to attain quantitative measurement and expression of buffer effects, and to derive from the mass law the above mentioned relationships.

The unit adopted is the differential ratio $\frac{dB}{dpH}$, expressing the relationship between the increment (in gram equivalents per liter) of strong base B added to a buffer solution and the resultant increment in pH. Increment of strong acid is equivalent to a negative increment of base, or $-dB$. In these terms *a solution has a buffer value of 1 when a liter will take up 1 gram equivalent of strong acid or alkali per unit change in pH.*

If base is added to a solution, pH is increased, so that both dB and dpH are positive. If acid is added both dB and dpH are negative. The ratio $\frac{dB}{dpH}$ is, therefore, always a positive numerical value. If one solution has twice the buffer value of a second solution, twice as much base or acid is required to change the pH by a given small amount, for example, 0.1, and therefore the value $\frac{dB}{dpH}$ is twice as great in the first solution as in the second. For convenience we shall use at times the letter β to indicate the ratio $\frac{dB}{dpH}$.

The significance of the ratio $\frac{dB}{dpH}$ as a measure of buffer effect is illustrated by Fig. 1. In place of dB and dpH, infinitesimal

sociation constant of the buffer acid [Ha], γ the degree of dissociation of its salt [Ba] into $[B^+]$ and $[a']$ ions. Hasselbalch actually used the form K_1 . We have altered it to K' in order to facilitate differentiating between K_a and K'_b , derived from the acid and basic dissociation constants customarily designated as K_a and K_b respectively.

increments, we there use measurable increments ΔB and ΔpH , which, if not too great, serve our purpose nearly as well. In order to increase the pH of Solution 1 from 3 to 4, 0.1 gram mole-

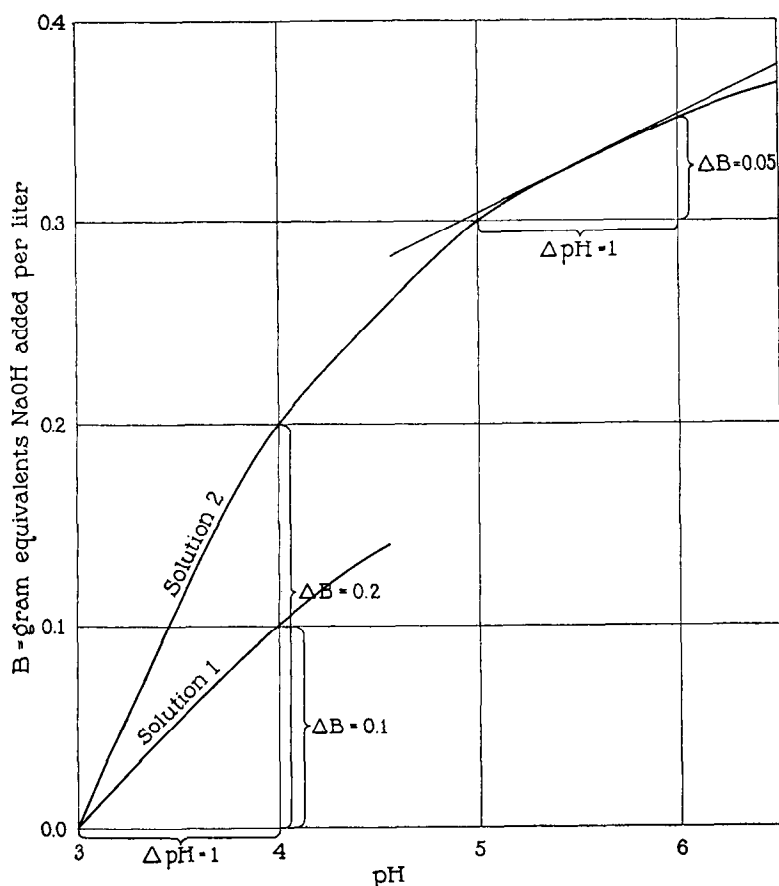


FIG. 1.

cule of NaOH per liter is required; therefore $\Delta B = 0.1$. Since $\Delta pH = 1$ (the increase in pH caused by the change from pH 3 to 4), the approximate buffer value of Solution 1 at the mean reaction of pH 3.5 is $\beta = \frac{\Delta B}{\Delta pH} = \frac{0.1}{1.0} = 0.1$.

In order to increase the pH of Solution 2 from pH 3 to pH 4, 0.2 gram molecule of NaOH per liter is required; therefore, for $\Delta\text{pH} = 1$, $\Delta\text{B} = 0.2$; and, at mean pH 3.5, $\beta = \frac{0.2}{1.0} = 0.2$.

Let us now follow the curve of Solution 2 further along until it covers the range of pH 5 to 6. Because the curve has become less steep, we find that the ΔB accompanying the pH increase from 5 to 6 is only 0.05. Consequently, Solution 2, which at pH 3.5 had a buffer value of $\frac{\Delta\text{B}}{\Delta\text{pH}} = 0.2$, has at pH 5.5 only one of $\frac{\Delta\text{B}}{\Delta\text{pH}} = 0.05$.

In place of measuring the ΔB and ΔpH values for equal distances on both sides of the pH at which we wish to determine the value of $\frac{dB}{dpH}$ we may draw a tangent to the curve at the indicated pH and measure the slope of the tangent. Thus, in the case of Solution 2, we may draw a tangent to the curve at pH 5.5. Since the tangent, being a straight line, has the same slope at all points, we may prolong it as much as we wish in order to determine its slope conveniently. For example, we make the measurements between pH 5 and 6.5. At these points the tangent cuts the B lines at 0.303 and 0.376 respectively, the difference being 0.073.

The slope of the tangent, or the value of $\frac{dB}{dpH}$ at pH 5.5, is therefore $\frac{0.073}{1.500} = 0.0486$, which approximates the value $\frac{\Delta\text{B}}{\Delta\text{pH}} = 0.05$ obtained above. The tangent, if accurately drawn, gives the exact $\frac{dB}{dpH}$ value at its point of contact with the curve, and there-

fore has some theoretical advantage over the use of the measurable increments ΔB and ΔpH , which indicate the approximate buffer value at the mean pH over the portion of curve measured.

The curve of Solution 2 illustrates the fact that a given solution may have a different buffer value at different reactions, and that one may speak in exact terms only of its buffer value at a definite pH, or of its average buffer value over a certain pH range. The manner in which the buffer value of a solution containing a single buffer acid and its salt changes with varying pH is shown by Figs. 4 and 9.

Since a solution may have a different buffer value at every pH, rigid accuracy would require that it be measured by the ratio $\frac{dB}{dpH}$, of the infinitesimal increments dB and dpH. Actually, as may be seen from Fig. 4, increments ΔB and ΔpH of sufficient size to be measured may be usually employed with fair accuracy.

The Buffer Value of Water Plus Only Strong Acid or Alkali.

When strong acid or alkali is added to water the solution displays a certain buffer value. If it did not, each addition of acid or alkali would cause infinite change in pH.

Let us assume that a strong base completely dissociating into the ions $[B^+]$ and $[OH']$ is added to water. Then the increment dB, in base, is equal to the increment $d[OH']$, in $[OH']$. Consequently for $\frac{dB}{dpH}$ we may write $\frac{d[OH']}{dpH}$. $pH = -\log [H^+] = -\log \frac{k_w}{[OH']} = \log [OH'] - \log k_w$, when k_w is the water constant $[OH'] \times [H^+]$. Hence $dpH = d \log [OH']$. Therefore

$$(1) \quad \frac{dB}{dpH} = \frac{d[OH']}{d \log [OH']} = \frac{[OH']}{0.4343} = 2.3 [OH']$$

Similarly, if we add to water a strong acid, completely dissociated into $[H^+]$ ions and anions, such addition constitutes a negative addition of base. The increment in acid is equal to that in $[H^+]$, that is, $-dB = d[H^+]$.

$$(2) \quad \frac{dB}{dpH} = \frac{-d[H^+]}{-d \log [H^+]} = \frac{[H^+]}{0.4343} = 2.3 [H^+]$$

If we add the two effects, expressed by Equations 1 and 2, respectively, we express the total buffer value of water plus completely dissociated acid or alkali at all pH's, as shown in Equation 3.

$$(3) \quad \frac{dB}{dpH} = 2.3 ([H^+] + [OH'])$$

At any given $[H^+]$ or $[OH']$, for each gram equivalent of hydron or hydroxyl ion present, completely dissociating base or acid must be added at the rate of 2.3 gram equivalents per liter per unit change in pH effected.

The relationships expressed in Equation 3 are shown in Figs. 2 and 3. It is obvious from them that over the reaction range usually significant in animal or plant physiology, pH 3 to 11, the buffer value of completely dissociated acid or alkali is slight, too slight in fact to show graphically on charts of the scale used, but that at both ends of this range it becomes significant.

The actual dissociation of a strong acid, such as HCl, or a strong base, such as NaOH, is only approximately complete at finite dilutions. If we express the fraction of BOH dissociated into

$$[B^+] \text{ and } [OH'] \text{ by } \gamma_B, [OH'] = \gamma_B [BOH] = \gamma_B B, B = \frac{[OH']}{\gamma_B},$$

and $dB = \frac{d[OH']}{\gamma_B}$ Similarly $dB = \frac{-d[H^+]}{\gamma_A}$ if γ_A represents the degree of dissociation of the strong acid. Consequently, if instead of ideal, completely dissociated strong base and acid, our additions to water are of actual, not quite completely dissociated strong base and acid, γ_B and γ_A being a little less than 1, instead of Equation 3, we have the slightly different Equation 4.

$$(4) \quad \frac{dB}{dpH} = 2.3 \left(\frac{[H^+]}{\gamma_A} + \frac{[OH']}{\gamma_B} \right)$$

The fact that this equation is accurate is illustrated by the following example. 0.1 N HCl is, according to the conductivity data of Noyes and Falk, 92.3 per cent dissociated at 20°. $[H^+] = 0.0923$ N; pH = 1.035. If we increase the HCl to 0.11 N, $[H^+]$ increases to $0.0923 \times 1.10 = 0.1015$ (the dissociation does not change significantly), and pH falls to 0.994. From these measured changes, ΔB and ΔpH , we calculate the approximate β value at the mean pH between 1.035 and 0.994.

$$\beta = \frac{\Delta B}{\Delta pH} = \frac{0.10 - 0.11}{0.994 - 1.035} = \frac{-0.01}{-0.041} = 0.243$$

The mean pH of the range, pH 1.035 to 0.994, is 1.015. The corresponding $[H^+]$ is 0.0966 ($[OH']$ is negligible). The buffer value of the solution, at pH 0.994, calculated by Equation 4, with $\gamma_A = 0.923$, is

$$\beta = \frac{dB}{dpH} = 2.3 \frac{0.0966}{0.923} = 0.241$$

Negelecting γ_A and calculating by Equation 3 we would obtain

$$\beta = 2.3 \times 0.0966 = 0.222$$

For HCl solutions more dilute than 0.1 N, γ_A approaches more nearly 1, and the difference between results calculated by Equations 3 and 4, respectively, becomes still less than in this example.

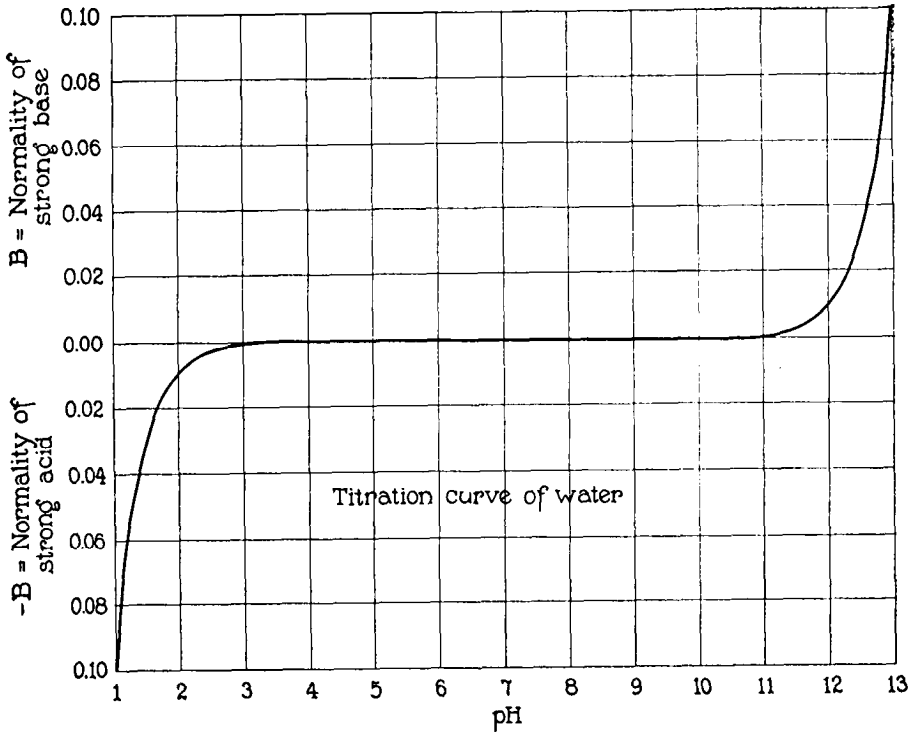


FIG. 2.

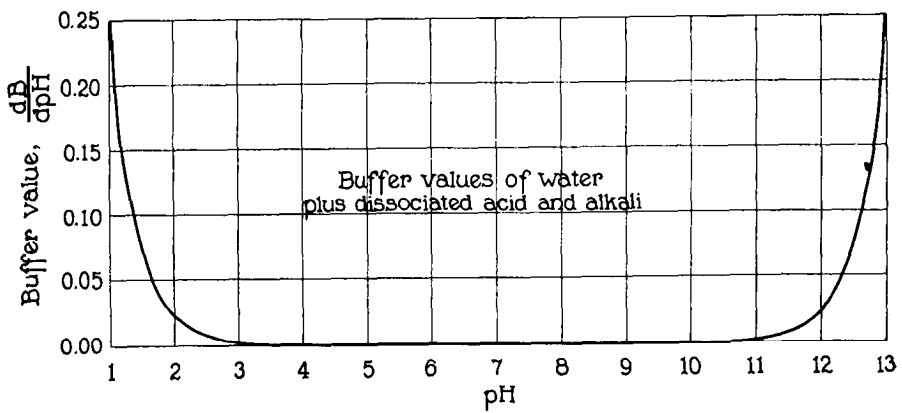


FIG. 3.

The buffer action of a strong, completely dissociated acid or base is different in its mechanism from the buffer action of a weak, slightly dissociated acid or base, such as was discussed in the first section of this paper, and is about to be considered quantitatively in the next section. The buffer action of the weak acid or base involves repression of part of the $[H^+]$ change that would be caused by addition of acid or alkali to an unbuffered solution. With repression of $[H^+]$ change there is also, of course, an accompanying repression of the *proportion* by which $[H^+]$ is altered, and it is this proportional $[H^+]$ change that is a linear function of the $\log [H^+]$ or pH change which we have adopted to measure buffer effect.

The fact that changes in $\log [H^+]$, and in pH, are linear measures of the proportion by which $[H^+]$ is changed may be demonstrated as follows. If $d [H^+]$ is the increment in $[H^+]$, the proportion of itself by which $[H^+]$ is increased is $\frac{d [H^+]}{[H^+]}$. If an increment, $-dB$, of strong acid is added,

with resultant absolute change in $[H^+]$ of $d [H^+]$, we have $\frac{-dB}{\frac{d [H^+]}{[H^+]}}$ as the

ratio between added acid and the proportion by which $[H^+]$ is increased. But in general,

$$\frac{dx}{\frac{dy}{y}} = \frac{dx}{d \log_e y} = \log_{10} e \frac{dx}{\log_{10} y}$$

$$\text{Hence } \frac{-dB}{\frac{d [H^+]}{[H^+]}} = -\log_{10} e \frac{dB}{\log_{10} [H^+]} = 0.4343 \frac{dB}{dpH}$$

$$dpH = -0.4343 \frac{d [H^+]}{[H^+]}$$

It is obvious from the right-hand member of the last equation that as factors of dpH we must consider not only $d[H^+]$, but also $[H^+]$.

The presence of a completely dissociated acid or base, would repress *only* the proportion by which a given further addition would alter the $[H^+]$. The absolute $[H^+]$ change would be practically unaffected by strong acid already present before this addition: only the $[H^+]$ factor of the $\frac{d[H^+]}{[H^+]}$ ratio would be affected.

The greater the amount of $[H^+]$ or $[OH']$ (or completely dissociated acid or alkali) present, however, the greater the further amount required to be added or removed to change the original to 10-fold or $\frac{1}{10}$ its own value, and thereby cause unit change in pH. And this further amount is our measure of buffer value. So, if we adopt pH change as our unit of reaction shift, we must assign a definite buffer effect to the mere mass of $[H^+]$ or $[OH']$ ions present, which, by its inertia, so to speak, represses pH change. This effect we may term *the buffer effect of the dissociated base or acid, or of the $[H^+]$ or $[OH']$ concentration*.

In a sense, therefore, the adoption of $\frac{dB}{dpH}$ as the unit of buffer value has broadened the conception of buffer action outlined in the introduction. Buffer actions have there been discussed as processes repressing $[H^+]$ changes by replacement of the added, more or less strongly dissociated acids or bases with the weak, undissociated acids or bases of the buffers. To this action we must add the "inertia" buffer effect peculiar to dissociated acids and bases, which repress the proportional, though not the absolute, $[H^+]$ change, and which attain importance when $[H^+]$ or $[OH']$ exceeds about 10^{-3} (when pH is less than 3 or greater than 11). As will appear later (Equations 17 and 35) the total buffer value of a solution is represented by the sum of the buffer effects due, respectively, to dissociated and to undissociated acids or bases present. The two effects may both be important in a buffer forming a very acid solution, such as H_3PO_4 , or forming a highly alkaline one, such as Na_3PO_4 .

The Buffer Value of a Solution of a Weak Monovalent Acid and Its Salt.

Fundamental Mass Law Equations.—The fundamental equation derived from the mass action law of Guldberg and Waage as applied by Arrhenius to the dissociation of electrolytes, and shown by Ostwald (1888) to hold for dilute solutions of weak acids is

$$(5) \quad K_a = \frac{[H^+] \times [a']}{[Ha]}$$

where $[Ha]$ is the undissociated portion of the acid, $[H^+]$ the hydron concentration, $[a']$ the concentration of anions formed by

dissociation of the acid, and K_a the dissociation constant of the acid.

This equation has been applied in two forms. It was used by Ostwald (1888) for pure solutions of weak acids in the form

$$(6) \quad K_a = \frac{[H^+]^2}{[Ha]}$$

The theoretical accuracy of Equation 6 for such solutions follows from the fact that both hydrions and anions must arise in equal numbers from the dissociation of $[Ha]$, when the latter is their sole significant source.

For solutions containing not only the free acid, but also its salt, $[Ba]$, Equation 5 was converted by L. J. Henderson (1908, 1909) into the form

$$(7) \quad K_a = \frac{[H^+] \gamma_s [Ba]}{[Ha]}$$

$[Ha]$ = concentration of free undissociated acid.

$[Ba]$ = total concentration of salt, dissociated or undissociated.

γ_s = fraction of $[Ba]$ dissociated into $[B^+]$ and $[a']$.

$\gamma_s[Ba]$ = concentration of a' formed by dissociation of $[Ba]$.

The salt is usually 80 per cent or more dissociated ($\gamma_s = 0.8$ to 1.0) while the free acid is dissociated to a relatively negligible extent into $[H^+]$ and $[a']$. The slight amount of $[a']$, equal to the $[H^+]$, arising from dissociation of the free acid is, at reactions covering a considerable zone on both sides of neutrality, negligible in comparison to the amount formed from the salt. Consequently, over a pH range of about 7 ± 3 or 4, $\gamma_s[Ba]$ can be inserted, with a negligible error in place of $[a']$ in Equation 5. (A certain inconsistency in symbols occurs through the use of $[Ha]$ to represent only the undissociated part of the acid, while $[Ba]$ represents the total salt, dissociated and undissociated. It has seemed preferable, however, to retain $[Ba]$ with this significance, partly because it has been used in this manner in the literature since Henderson introduced his equation, partly because it has the advantage of being a self-explanatory symbol.)

Hasselbalch (1917) expressed Henderson's equation in the logarithmic form

$$(8) \quad pH = pK'_a + \log \frac{[Ba]}{[Ha]}$$

pK'_a being the negative logarithm of K_a , while $K'_a = \frac{K_a}{\gamma_s}$.

Equation 7, and its equivalent, Equation 8, hold for all $[H^+]$ values that are found in body fluids and excretions (except perhaps gastric juice) since the conditions are fulfilled that $[H^+]$ and $[OH']$ are both small (in the neighborhood of 10^{-7} in the internal fluids, and not exceeding 10^{-5} in urine), and the concentration of anions formed by dissociation of the buffer salts present is sufficient to make $\gamma_s [Ba]$ the only factor that requires consideration in estimating $[a']$. Equations 7 and 8 are therefore accurate for the physiological range for which Henderson used them, and may in fact be used over a still wider reaction range. The limitations of this range will be considered later.

Differentiation of Henderson's Equation in Order to Calculate the Buffer Values of Weak Acids.—In order to obtain the buffer value, $\frac{dB}{dpH}$, in terms of the buffer concentration and reaction, we convert Equation 7 into the form

$$(9) \quad K_a = \frac{[H^+] \gamma_s B}{C - B}$$

C = total molecular concentration of buffer acid before addition of base.

B = gram equivalents of strong base added.

$C - B = [HA]$

Within the range of validity of Henderson's equation, all the BOH added takes the form of the buffer salt, $[Ba]$, the base remaining free as BOH being negligible. Therefore, we may substitute B for $[Ba]$ in Equation 7. Furthermore, since all save a negligible portion of the buffer acid is in the form either of free undissociated acid, $[Ha]$, (the negligible residue is dissociated into $[H^+]$ and $[a']$), or salt, $[Ba]$, we may substitute $C - B$ for $[Ha]$. With these two substitutions Equation 7 is changed into Equation 9.

Solving Equation 9 for B we have

$$(10) \quad B = \frac{K_a C}{K_a + \gamma_s [H^+]} = \frac{K'_a C}{K'_a + [H^+]} \quad (\text{where } K'_a \gamma_s = K_a)$$

$$\frac{dB}{dpH} = - \frac{dB}{d \log [H^+]} = - \frac{[H^+]}{0.4343} \times \frac{dB}{d[H^+]} = - 2.3 [H^+] \frac{dB}{d[H^+]}$$

Differentiating the first form of Equation 10, $B = \frac{K_a C}{K_a + \gamma_s [H^+]}$, we have

$$\frac{dB}{d[H^+]} = - \frac{K_a \gamma_s C}{(K_a + \gamma_s [H^+])^2}$$

From which we obtain, by multiplying $\frac{dB}{d[H^+]}$ by $-2.3 [H^+]$,

| | | |
|------|--|---|
| (11) | $\frac{dB}{dpH} = \frac{2.3 K_a \gamma_s C [H^+]}{(K_a + \gamma_s [H^+])^2}$ | $\left. \begin{array}{l} \text{Equivalent} \\ \text{expressions} \\ \text{for the buffer} \\ \text{value } \frac{dB}{dpH}, \\ \text{or } \beta, \\ \text{for weak acids.} \end{array} \right\}$ |
| (12) | $= \frac{2.3 K'_a C [H^+]}{(K'_a + [H^+])^2}$ | |
| (13) | $= \frac{2.3 [Ba] [H^+]}{(K'_a + [H^+])}$ | |
| (14) | $= \frac{2.3 [Ba] [Ha]}{C}$ | |

Equation 12 is derived from Equation 11 by substituting $K'_a \gamma_s$ for its equivalent K_a , or by differentiation of the second form of Equation 10, in which this substitution is already made. Equation 13 is derived from Equation 12 by substituting $[Ba]$ for

$\frac{K'_a C}{K'_a + [H^+]}$. (Henderson's equation may be written $K'_a (C - [Ba]) = [H^+] [Ba]$, whence $[Ba] = \frac{K'_a C}{K'_a + [H^+]}$). Equation 14

is derived from Equation 13 by substituting $[Ha]$ for $\frac{[H^+] C}{K'_a + [H^+]}$.

(If Henderson's equation is written $K'_a [Ha] = [H^+] (C - [Ha])$ it yields this value for $[Ha]$).

We have tested the approximate accuracy of Equation 14 (and therefore of its equivalents, Equations 11, 12, and 13) against experimental results by applying it to Clark and Lubs' data for $KH_2PO_4 - KHPO_4$ mixtures (1916), the pH values being electrometrically determined by Clark for varying $[Ba]:[Ha]$, or $K (KHPO_4) : H (KH_2PO_4)$, ratios. Considering the short intervals used ($\Delta pH = 0.2$), the agreement between the theoretically calculated $\frac{dB}{dpH}$ values and those approximated from the

experimental ΔB and ΔpH figures is as satisfactory as could be expected from this mode of calculation.

The data of Table I are expressed graphically in Fig. 4, the curve being calculated from Equation 8 with $pK'_a = 6.85$. Clark's experimentally determined points are indicated by crosses.

It is evident from Equation 12 that the buffer effect expressed in terms of K_a and $[H^+]$ is proportional to the total molecular concentration $[C]$ of the buffer. We may therefore divide the derivative by $[C]$, in order to determine the *molecular buffer value* of a solution, or the buffer value which an M solution of it would have. This unit may be used for comparison of different buffers. It becomes

$$(15) \quad \frac{dB}{CdpH} = \frac{2.3 K'_a [H^+]}{([H^+] + K'_a)^2} = \beta_M$$

We shall hereafter refer for convenience to the absolute buffer value as β , the molecular buffer value as β_M . The relationship between the two is

$$(16) \quad \frac{\beta}{C} = \beta_M$$

The total buffer value of a solution of a weak acid to which both strong acid and alkali are added in amounts not limited to equivalence with the buffer acid, may be expressed as the β , of the buffer plus the β of the free, dissociated acid or alkali. This is expressed by combining Equations 3 and 12 in Equation 17.

$$(17) \quad \beta = 2.3 \left(\frac{K'_a C [H^+]}{(K'_a + [H^+])^2} + [H^+] + [OH'] \right)$$

For acetic acid ($pK'_a = 4.6$) in 0.2 and 0.1 M concentrations, the total buffer value of the solution from pH 1 to 13 is indicated by Fig. 5. Where the buffer effects of the acetate and hydrion overlap, the separate β values are indicated by broken lines, the actual total β values of the solution by continuous lines.

The use of $\frac{\beta}{C}$ as β_M is obviously permissible only where $[H^+]$ and $[OH']$ are negligible in comparison with $\frac{K_a C [H^+]}{(K'_a + [H^+])^2}$ which is the case, with $C = 0.1 M$, between pH values of about 3 and 11.

TABLE I.

Values of $\frac{\Delta B}{\Delta pH}$, as Estimated from Values of ΔB and ΔpH Taken Directly from W. M. Clark's Curves for 0.05 M KH_2PO_4 Plus NaOH, Compared with Values of $\frac{dB}{dpH}$ Calculated by Equation 14.

| Phosphate mixtures from Clark. C = 0.05 M | | | | | | | | | | | |
|--|----------|-------------|--------|-----------------|------------|------------|------------|------------------------------|--|---------------------------------------|-----------------------|
| pH | Mean pH. | Δ pH | [Ba] | pK _a | Mean [Ba]. | Mean [Ha]. | Δ B | $\frac{\Delta B}{\Delta pH}$ | $\frac{dB}{dpH}$ calculated as 46 [Ha] [Ba] [†] from mean [Ha] and [Ba]. | Molecular buffer value β_M . | |
| | | | | | | | | | | $\frac{\Delta B}{0.05 \Delta pH}$ | $\frac{dB}{0.05 dpH}$ |
| 5.8 | | | 0.0037 | 6.90 | | | | | | | |
| | 5.9 | 0.2 | | | 0.0047 | 0.0453 | 0.0020 | 0.0100 | 0.0098 | 0.200 | 0.198 |
| 6.0 | | | 0.0057 | 6.89 | | | | | | | |
| | 6.1 | 0.2 | | | 0.0062 | 0.0438 | 0.0029 | 0.0145 | 0.0125 | 0.290 | 0.250 |
| 6.2 | | | 0.0086 | 6.88 | | | | | | | |
| | 6.3 | 0.2 | | | 0.0106 | 0.0394 | 0.0040 | 0.0200 | 0.0192 | 0.400 | 0.384 |
| 6.4 | | | 0.0126 | 6.87 | | | | | | | |
| | 6.5 | 0.2 | | | 0.0152 | 0.0348 | 0.0052 | 0.0260 | 0.0245 | 0.520 | 0.490 |
| 6.6 | | | 0.0178 | 6.86 | | | | | | | |
| | 6.7 | 0.2 | | | 0.0207 | 0.0293 | 0.0058 | 0.0290 | 0.0279 | 0.580 | 0.558 |
| 6.8 | | | 0.0236 | 6.85 | | | | | | | |
| | 6.9 | 0.2 | | | 0.0266 | 0.0234 | 0.0060 | 0.0300 | 0.0287 | 0.600 | 0.574 |
| 7.0 | | | 0.0296 | 6.84 | | | | | | | |
| | 7.1 | 0.2 | | | 0.0323 | 0.0177 | 0.0054 | 0.0270 | 0.0263 | 0.540 | 0.526 |
| 7.2 | | | 0.0350 | 6.83 | | | | | | | |
| | 7.3 | 0.2 | | | 0.0372 | 0.0123 | 0.0045 | 0.0225 | 0.0211 | 0.450 | 0.422 |
| 7.4 | | | 0.0395 | 6.83 | | | | | | | |
| | 7.5 | 0.2 | | | 0.0411 | 0.0089 | 0.0033 | 0.0165 | 0.0168 | 0.330 | 0.336 |
| 7.6 | | | 0.0428 | 6.83 | | | | | | | |
| | 7.7 | 0.2 | | | 0.0440 | 0.0060 | 0.0024 | 0.0120 | 0.0121 | 0.240 | 0.242 |
| 7.8 | | | 0.0452 | 6.83 | | | | | | | |
| | 7.9 | 0.2 | | | 0.0450 | 0.0050 | 0.0016 | 0.0080 | 0.0104 | 0.160 | 0.208 |
| 8.0 | | | 0.0468 | 6.83 | | | | | | | |

* Calculated as $pK'_a = pH - \log \frac{[Ba]}{0.05 - [Ba]}$.

† $\frac{2.3}{C} = \frac{2.3}{0.05} = 46$ (Equation 14).

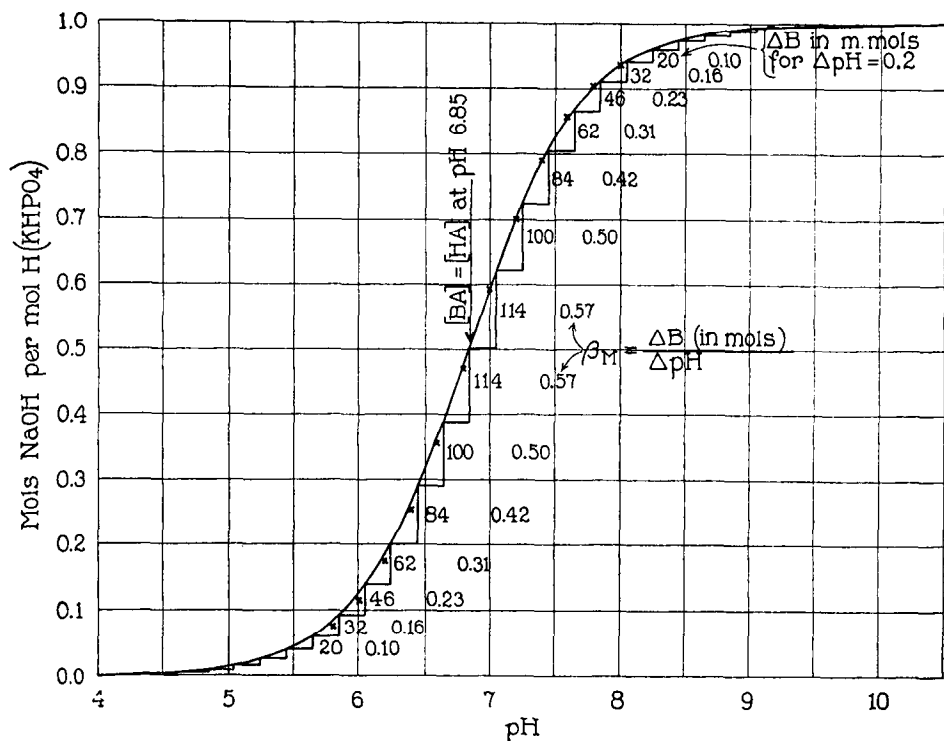


FIG. 4. Titration curve of H(KH₂PO₄) plus NaOH. Curve is calculated from equation, $\text{pH} = 6.85 + \log \frac{\text{Na(KH}_2\text{PO}_4)}{\text{H(KH}_2\text{PO}_4)}$. Crosses are from experimental data of Clark and Lubs.

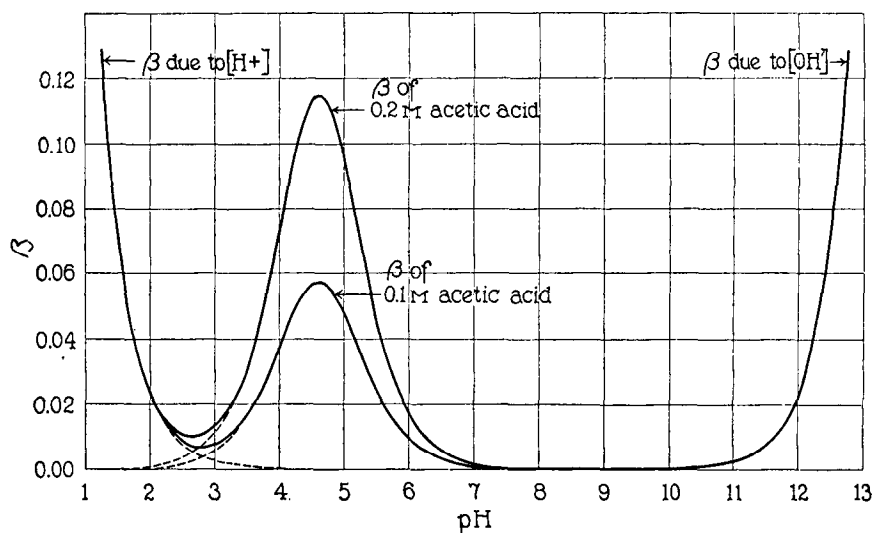


FIG. 5.

Second Derivative of Henderson's Equation. Point of Maximum Buffer Value.—To find the conditions under which a mixture of a weak acid and its salt exerts maximum buffer effect, we repeat the differentiation. From Equation 12, $\beta_m = 2.3 K'_a \frac{[H^+]}{K'_a + [H^+]}$

$$\frac{d\beta_m}{d[H^+]} = 2.3 K'_a \frac{[K'_a]^2 - [H^+]^2}{(K'_a + [H^+])^4} = 2.3 K'_a \frac{K'_a - [H^+]}{(K'_a + [H^+])^3}$$

$$(18) \quad \frac{d\beta_m}{dpH} = -2.3 [H^+] \frac{d\beta_m}{d[H^+]} = 2.3 K'_a [H^+] \frac{[H^+] - K'_a}{(K'_a + [H^+])^3}$$

From Equation 18 it is evident that β_m , the molecular buffer value, has either a maximum or a minimum value when $[H^+] = K'_a$, since this is the condition for making $\frac{d\beta_m}{dpH}$ equal to zero. The fact that maximum, not minimum, value of β_m occurs when $[H^+] = K'_a$ is obvious from an inspection of the curve of Fig. 4. The occurrence of the maximum at this point was somewhat less rigidly shown by the writer in a former paper (1921, a).

At the maximum point, since $[H^+] = K'_a$, we have from Equation 15

$$(19) \quad \beta_m = \frac{2.3 [H^+]^2}{(2[H^+])^2} = \frac{2.3}{4} = 0.575$$

For every buffer this slope indicates a value of $[Ha] = [Ba]$, since when $K'_a = [H^+]$, $[Ha]$ must equal $[Ba]$, as is evident when Henderson's equation is put into the form $[Ba] [H^+] = [Ha] K'_a$.

The relationships developed lead to the following conclusions, an example of each of which may be obtained from Table I and Fig. 4.

1. All monovalent acid buffers acting within the range of validity of Henderson's equation (with pK'_a between about 4 and 10) have the same maximum molecular buffer value, viz. $\beta_m = 0.575$, which they exert when $[H^+] = K'_a$, or $pH = pK'_a$. (In the case of $H(KHPO_4)$, used in Table I, $pK'_a = 6.85$.)

2. At this pH, $[Ba] = [Ha] = \frac{C}{2}$, that is, half the buffer acid is free, half in the form of alkali salt.

3. If the point of maximum slope be determined in a buffer curve, with increments of added alkali as ordinates, pH values

as abscissæ, one may calculate the molecular concentration of the buffer by Equation 20, which is a rearrangement of Equation 19.

$$(20) \quad C = \frac{\beta}{0.575}$$

i.e., the molecular concentration of the buffer is found by dividing the tangent of the maximum slope of the titration curve by 0.575.

Equation 19 holds even for polybasic acids, provided the dissociation constants of the different acid hydrogen atoms are far enough apart so that, at the pH of its maximum buffer efficiency, each one exerts a buffer effect relatively uninfluenced by the others. If two buffer acids are in equal concentration in the same solution, their pK' values must be at least 2.5 apart in order that the buffer effect of each may be less than 1 per cent that of the other at the latter's maximum. This is the case with phosphoric acid, of which the three pK' values are 2.0, 6.8, and 12.0, as seen from Fig. 6. The manner in which polybasic acids may be handled when their buffer effects overlap is discussed later.

It is to be noted that the relationship $[Ba] = [Ha]$ at a point of maximum buffer value in such a polyvalent acid buffer refers to $[Ba]$ only as the amount of base replacing the hydrogen of the particular acid group which is acting as a buffer at the existing pH.

4. The pH at the point of maximum slope of the buffer curve indicates the value of pK'_a .

5. For β values other than the maximum, K'_a may be calculated by solving Equation 12 for K'_a . We obtain thus from Equation 12

$$(22) \quad K'_a = [H^+] \frac{C - 0.8686 \beta_M + \sqrt{(0.8686 \beta_M - C)^2 - 0.756 \beta_M^2}}{0.8686 \beta_M}$$

Of the two K'_a values obtained by Equation 22, that is correct which is identical with $[H^+]$ at the point of maximum β value, or the β_M value of 0.575. While ordinarily there is no advantage in using values of β or β_M , and Equation 22, rather than values of $[Ba]$ and Equation 7 in calculating an acid dissociation constant, yet, there may be occasions in which β is more readily determinable than $[Ba]$, and in which, therefore, such an advantage exists.

The calculations indicated by Equation 22 may be performed graphically with the aid of the curve (Fig. 9) expressing values of $U (U = \frac{\beta_M}{0.575} = 1 \text{ for maximum } \beta_M)$ and of $\log \frac{[H^+]}{K'_a}$ (which is equal to $pK'_a - pH$) plotted by L. J. Henderson in the note forming part of this paper. By this curve, when β_M is known, the corresponding value of $pK'_a - pH$, and hence the value of pK'_a may be found by inspection. Likewise, when the pK'_a of an acid buffer is known, the β value at any pH may be found on the curve.

If instead of taking $\frac{dB}{dpH}$ as the unit of buffer value we used $\frac{dB}{d[H^+]}$, or, to obtain positive values, $\frac{dA}{d[H^+]}$ where $A = -B = \text{added strong acid}$, we would obtain

$$(a) \quad \frac{dA}{d[H^+]} = -\frac{dB}{d[H^+]} = \frac{CK'_a}{(K'_a + [H^+])^2}$$

The molecular buffer value in these terms would be

$$(b) \quad \frac{dA}{C d[H^+]} = \frac{K'_a}{(K'_a + [H^+])^2}$$

The second derivation is

$$(c) \quad \frac{d^2 A}{C d[H^+]^2} = -\frac{2K'_a}{(K'_a + [H^+])^3}$$

From Equation c it is apparent that there is no point of maximum $\frac{dA}{d[H^+]}$ value. The second derivative at no point changes through zero from a positive to a negative value or *vice versa*. It is always negative, showing that $\frac{dB}{d[H^+]}$ decreases continuously as long as $[H^+]$ has any finite value. For mixtures containing only the free buffer acid and its salt, without additional alkali, minimum $[H^+]$, and therefore maximum $\frac{dA}{d[H^+]}$ values, are obtained when all the acid is bound as salt. At this point $\frac{dA}{d[H^+]} = \frac{1}{K'_a}$. Yet experience has led experimenters to decide that weak acids act most efficiently as buffers when they are present about half as free acid, $[Ha]$, half as salt, $[Ba]$ (Clark,² 1920). For buffer mixtures containing equal parts of $[Ha]$ and $[Ba]$, the value of $\frac{dA}{d[H^+]}$ is $\frac{1}{4K'_a}$ (obtained

by substituting in Equation b $\frac{K'_a [\text{Ha}]}{[\text{Ba}]}$ for the value of $[\text{H}^+]$ obtained by solving for $[\text{H}^+]$ Henderson's equation $K'_a = \frac{[\text{H}^+][\text{Ba}]}{[\text{Ha}]}$. This means that, with half the buffer acid free in each case, half in the form of its salt, the $\frac{dA}{d[\text{H}^+]}$ values for the three phosphoric acids, $\text{H}(\text{H}_2\text{PO}_4)$, $\text{H}(\text{HKPO}_4)$, and $\text{H}(\text{K}_2\text{PO}_4)$, would be in round numbers, respectively, $\frac{1}{4 \times 10^{-2}}$, $\frac{1}{4 \times 10^{-7}}$, and $\frac{1}{4 \times 10^{-12}}$, or 25, 2,500,000, and 250,000,000,000. In the most alkaline of the three solutions, where $\text{H}(\text{K}_2\text{PO}_4) = \text{K}_3\text{PO}_4$, $\frac{dA}{d[\text{H}^+]}$ is enormous, merely because $[\text{H}^+]$ is infinitesimal and $d[\text{H}^+]$ is proportionally reduced. If we based the unit of buffer value on ability to minimize $[\text{OH}']$ instead of $[\text{H}^+]$ change, that is, on the value of $\frac{dB}{d[\text{OH}']}$, conditions would be reversed, and the buffer value of the alkaline phosphate mixture would be estimated as a billion times greater than that of the acid phosphate. If, to avoid this embarrassment, we used $\frac{dA}{d[\text{H}^+]}$ for the acid range, $\frac{dB}{d[\text{OH}']}$ for the alkaline, the acid and alkaline phosphate mixtures would each have similar buffer values, but 100,000 times greater than the neutral $\text{H}(\text{KHPO}_4) - \text{K}(\text{KHPO}_4)$ mixture. Similar huge ranges of figures are encountered if we use the unit $1 - \frac{d[\text{H}^+]}{dA}$.

These anomalies and inconveniences disappear when we use the logarithmic $\frac{dB}{dpH}$ unit. Each buffer in terms of this unit has the same maximum effect, $\frac{dB}{dpH} = 0.575 C$, when $[\text{Ha}] = [\text{Ba}]$, and each has within the zone where its effect is exerted, the same symmetrical curve of buffer value, the U-curve of Fig. 9.

For expressing reaction changes, Clark and Lubs (1916) have emphasized the advantages of Sørensen's pH unit, due to its avoidance of huge range of numbers, to the elegance with which pH data over wide ranges of reaction may be graphically plotted, to the fact that errors in our methods of determination are proportional to changes in pH rather than in $[\text{H}^+]$, and to the fact

that changes in pH rather than in $[H^+]$ appear to be proportional to changes in physiological effects.

For expressing buffer values the pH unit adds to these advantages that of yielding a uniform and symmetrical β curve for buffers, and a uniform maximum value.

The curve⁴ of Van Slyke and Zacharias (1914), showing the rate of action of urease in M/11 phosphate at varying pH, affords an illustration of the parallelism between pH change (or proportional $[H^+]$ change) and effect on an enzymic activity, and of the relative lack of parallelism between absolute $[H^+]$ change and effect on activity. In Table II. are indicated the pH and $[H^+]$ changes accompanying the same decrease in rate of enzyme action on both sides of the optimum pH. Approximately the same pH

TABLE II.

| | Acid side of optimum. | Alkaline side of optimum. |
|--|------------------------|---------------------------|
| pH when urease reaction rate = 0.4..... | 6.25 | 8.47 |
| " " " " = 0.3..... | 5.80 | 8.90 |
| " change..... | -0.45 | +0.43 |
| $[H^+]$ when reaction rate = 0.4..... | 5.6×10^{-7} | 0.034×10^{-7} |
| " " " " = 0.3..... | 15.9×10^{-7} | 0.012×10^{-7} |
| " change..... | $+10.3 \times 10^{-7}$ | -0.022×10^{-7} |
| Mean $[H^+]$ | 10.7×10^{-7} | 0.023×10^{-7} |
| Proportion by which $[H^+]$ is changed = $\frac{[H^+]\text{ change}}{\text{mean } [H^+]}$ | +0.96 | -0.96 |

change, positive on one side, negative on the other, accompanies the same decrease in rate of enzyme action on both the alkaline and acid sides. It follows that the proportions, +0.96 and -0.96, by which $[H^+]$ is changed are (in this case exactly) equal. But the absolute $[H^+]$ change on the acid side, 10.3×10^{-7} is about 500 times that (0.02×10^{-7}) required on the alkaline side to cause the same decrease in rate of enzyme action. The parallelism between pH change and effect on biological reactions is not, of course, regularly as accurate as happens to be the case in this example. But it appears to be true that rates of enzyme action (and most reactions in the organism appear to be enzymic) as a rule form fairly symmetrical curves when plotted against pH or log $[H^+]$, with some degree of quantitative parallelism on both sides of the optimum between pH change and its effect; while there is no such approach to parallelism between effect and absolute $[H^+]$ change.

⁴ Van Slyke and Zacharias (1914), p. 194.

General Equation Indicating the Dissociation of a Weak Acid at All Reactions within the Limits of Validity of the Mass Law.—As stated above Henderson's equation is valid only within a range of reaction which is ordinarily 7 ± 3 or 4, and in consequence the equations expressing the values of β that have been derived above by differentiation of Henderson's equation are subject to the same limitation. In order to express the relationships of β to the hydron concentration of the solution, and the concentration and dissociation constant of the buffer acid, in an equation valid for as great a range of conditions as that limiting the validity of the mass law for dilute acids, we must derive a form of Equation 5 that is general for mixtures of weak acids and their salts, and not limited to the condition that $[H^+]$ and $[OH']$ shall be negligible in comparison with C and [Ba]. In order to obtain the desired equation in a form that can be differentiated for calculation of $\frac{dB}{dpH}$ it is necessary furthermore to express the values of $[a']$ and [Ha] in terms of C and B.

Let C = total molecular concentration of buffer acid, in form of either free acid or its salt.

B = concentration of strong base BOH added to acid (gram equivalents of base per liter of solution).

[Ba] = concentration of salt Ba.

[Ha] = concentration of undissociated free buffer acid.

$[a']$ = concentration of anion formed by dissociation of the acid Ha and the salt Ba.

γ_s = fraction of buffer salt Ba dissociated into B^+ and a' ions.

γ_b = fraction of free strong base BOH dissociated into B^+ and OH' (BOH is, of course, present only in alkaline solutions such as that of Na_3PO_4).

We wish to ascertain general values of $[A']$ and [Ha] to insert in the basic Equation 5, $K_a[Ha] = [H^+][a']$. The value of $[a']$ may be ascertained by equating the concentrations of all the negative and positive ions, respectively.

$$\begin{aligned}
 [B^+] + [H^+] &= [a'] + [OH'] \\
 [a'] &= [B^+] + [H^+] - [OH'] \\
 &= [B^+] + [H^+] - \frac{K_w}{[H^+]}
 \end{aligned}
 \tag{23}$$

The terms constituting functions of $[H^+]$ in the right-hand member we may use as they are. $[B^+]$, however, we must obtain in terms of the total base added, B , and of $[H^+]$.

$$(24) \quad [B^+] = \gamma_s [Ba] + \gamma_b [BOH]$$

$$[BOH] = \frac{[OH']}{\gamma_b} = \frac{K_w}{\gamma_b [H^+]}$$

$$[Ba] = B - [BOH]$$

$$(25) \quad = B - \frac{K_w'}{\gamma_b [H^+]}$$

Substituting in Equation 24 for $[BOH]$ its equivalent as given above, and for $[Ba]$ its equivalent from Equation 25 we have

$$\begin{aligned} [B^+] &= \gamma_s \left(B - \frac{K_w}{\gamma_b [H^+]} \right) + \frac{K_w}{[H^+]} \\ &= \gamma_s B + \left(1 - \frac{\gamma_s}{\gamma_b} \right) \frac{K_w}{[H^+]} \end{aligned}$$

Substituting the above value for $[B^+]$ in Equation 23 we have

$$[a'] = \gamma_s B + \left(1 - \frac{\gamma_s}{\gamma_b} \right) \frac{K_w}{[H^+]} + [H^+] - \frac{K_w}{[H^+]}, \text{ or}$$

$$(26) \quad [a'] = \gamma_s B + [H^+] - \frac{\gamma_s K_w}{\gamma_b [H^+]}$$

The concentration of undissociated free buffer acid, $[Ha]$, we find by subtracting from the total buffer concentration, C , the part, represented by $[Ba]$, in the form of salt, and the part, equal to $[H^+]$, in the form of free acid dissociated according to the equation $[Ha] = [H^+] + [a']$

$$[Ha] = C - [Ba] - [H^+]$$

Substituting for $[Ba]$ its value, as found above in Equation 25, we have

$$(27) \quad [Ha] = C - B + \frac{K_w}{\gamma_b [H^+]} - [H^+]$$

Substituting in our basic Equation 5 the above values for $[a']$ and $[Ha]$ gives us

$$(28) \quad K_a = \frac{[H^+] \left(\gamma_s B - \frac{\gamma_s [OH']}{\gamma_B} + [H^+] \right)}{C - B + \frac{[OH']}{\gamma_B} - [H^+]}$$

Substituting $K'_a \gamma_s$ for K_a , and dividing both sides of the equation by γ_s , we obtain

$$(29) \quad K'_a = \frac{[H^+] \left(B - \frac{[OH']}{\gamma_B} + \frac{[H^+]}{\gamma_s} \right)}{C - B + \frac{[OH']}{\gamma_B} - [H^+]}$$

With the introduction of but slight error we may simplify Equation 29 by substituting 1 for γ_B , since, in solutions more dilute than 0.01 N, NaOH and KOH approach complete dissociation. We may also, in the term $\frac{[H^+]}{\gamma_s}$ substitute 1 for γ_s , since in weak buffer acid-salt solutions of such high acidity that $[H^+]$ is an important factor in the equation, the ratio $\frac{[Ha]}{[Ba]}$ is sure to be high, with $[Ba]$ correspondingly dilute, and γ_s approaching 1 in consequence. Thus simplified Equation 29 becomes

$$(30) \quad K'_a = \frac{[H^+] (B + [H^+] - [OH'])}{C - (B + [H^+] - [OH'])}$$

In solutions of free acids, $B = 0$ and $[OH']$ is negligible. Equation 30 then becomes $K_a = \frac{[H^+]^2}{C - [H^+]}$, which is equivalent to Ostwald's dilution law (Equation 6) since, with only free acid present, $C - [H^+] = [Ha]$. In a solution containing both buffer salts and free acid, and with $[H^+]$ and $[OH']$ both insignificant in comparison with B (solution bordering neutrality), Equation 30 becomes $K'_a = \frac{[H^+]}{C - B} \frac{B}{B}$, which is equivalent to Henderson's equation (Equation 7). Both Ostwald's and Henderson's equations are, therefore, special cases of Equation 30.

In acid and near-neutral solutions ($\text{pH} < 10$), $[\text{OH}']$ becomes negligible and Equation 30 becomes

$$(31) \quad K'_a = \frac{[\text{H}^+](B + [\text{H}^+])}{C - (B + [\text{H}^+])}$$

In alkaline and near-neutral solutions ($\text{pH} > 4$) it becomes

$$(32) \quad K'_a = \frac{[\text{H}^+](B - [\text{OH}'])}{C - (B - [\text{OH}'])}$$

We may test the approximate accuracy of Equation 30 in the acid and alkaline range by applying it to the titration curves of W. M. Clark⁵ (1920), for $\text{H}_3\text{PO}_4 + \text{KOH}$ and for $\text{H}(\text{K}_2\text{PO}_4) + \text{KOH}$. (For the neutral range it has already been tested in the form of Henderson's equation in Table I.) Clark titrated 50 cc. of 0.1 M (or 0.3 N) H_3PO_4 with 150 cc. of 0.1 N KOH , and determined the pH curve. The volume was, therefore, increasing throughout the titration. From Clark's curves we obtain the data of Table III. The published curves are drawn on a scale which limits pH readings to an accuracy of about 0.05, but this suffices, considering the wide range covered, to permit use of the data to test the approximate accuracy of Equation 30. As seen from the last 2 columns, the equation gives a true constant.

In order to differentiate Equation 30 we substitute $\frac{K_w}{[\text{H}^+]}$ for $[\text{OH}']$, and K_w for the term $[\text{H}^+][\text{OH}']$, solve the equation for B, and obtain

$$\begin{aligned} B &= \frac{K'_a C [\text{H}^+] - K'_a [\text{H}^+]^2 - [\text{H}^+]^3 + K_w K'_a + K_w [\text{H}^+]}{[\text{H}^+](K'_a + [\text{H}^+])} \\ &= \frac{[\text{H}^+] K'_a C}{[\text{H}^+](K'_a + [\text{H}^+])} - \frac{[\text{H}^+]^2 (K'_a + [\text{H}^+])}{[\text{H}^+](K'_a + [\text{H}^+])} + \frac{K_w (K'_a + [\text{H}^+])}{[\text{H}^+](K'_a + [\text{H}^+])} \\ (33) \quad &= \frac{K'_a C}{K'_a + [\text{H}^+]} - [\text{H}^+] + \frac{K_w}{[\text{H}^+]} \end{aligned}$$

$$\begin{aligned} (34) \quad \frac{dB}{d[\text{H}^+]} &= -\frac{K'_a C}{(K'_a + [\text{H}^+])^2} - 1 - \frac{K_w}{[\text{H}^+]^2} \\ \frac{dB}{dpH} &= -2.3 [\text{H}^+] \frac{dB}{d[\text{H}^+]} \end{aligned}$$

$$(35) \quad \beta = \frac{dB}{dpH} = 2.3 \left(\frac{K'_a C [\text{H}^+]}{(K'_a + [\text{H}^+])^2} + [\text{H}^+] + [\text{OH}'] \right)$$

⁵ Clark (1920), p. 32.

TABLE III.
Calculation of K'_a Values of $H_3PO_4 - KH_2PO_4$ and of $HK_2PO_4 - K_3PO_4$ Mixtures by Means of Equation 30.

| Mixtures present. | 0.1 N KOH added to 50 cc. 0.1 M H_3PO_4 . | V total volume of solu- tion. | C $= \frac{0.1 \times 50}{V}$ | B $= \frac{0.1 \times \text{cc. KOH}}{V}$ | pH | $[H^+]$ | $[OH^-]$ | K'_a | $pK'_a =$ $-\log K'_a$ |
|-------------------------------------|---|--|----------------------------------|--|-------|-----------------------|----------|-------------------------|---------------------------|
| $H_3PO_4 -$ KH_2PO_4 | cc. | cc. | N | N | | | N | | |
| | 0 | 50 | 0.1000 | 0.0000 | 1.60 | 0.0251 | 0.0000 | 0.84×10^{-2} * | 2.08 |
| | 10 | 60 | 0.0834 | 0.0167 | 1.80 | 0.0159 | 0.0000 | " | 2.00 |
| | 20 | 70 | 0.0714 | 0.0286 | 2.00 | 0.0100 | 0.0000 | " | 1.93 |
| | 30 | 80 | 0.0625 | 0.0375 | 2.30 | 0.0050 | 0.0000 | " | 1.97 |
| | 40 | 90 | 0.0556 | 0.0444 | 2.70 | 0.0020 | 0.0000 | " | 2.00 |
| Average of last 4 calculations..... | | | | | | | | | |
| $HK_2PO_4 -$ K_3PO_4 | 100 + 10 | 160 | 0.0313† | 0.0062 | 11.20 | 6.31×10^{-3} | 0.0016 | 1.10×10^{-2} | 11.96 |
| | 100 + 20 | 170 | 0.0294 | 0.0118 | 11.55 | 2.82 " | 0.0036 | " | 11.97 |
| | 100 + 30 | 180 | 0.0278 | 0.0168 | 11.80 | 1.59 " | 0.0063 | " | 12.02 |
| | 100 + 40 | 190 | 0.0263 | 0.0211 | 11.95 | 1.12 " | 0.0089 | " | 12.01 |
| Average | | | | | | | | | 11.99 |

* The first figures, 0.84×10^{-2} and 2.08, really represent K_a rather than K'_a , since there is present only free acid without salt. Since $K_a = \gamma_s K'_a$, if γ_s in the other determinations has a value of 0.8, they would yield an average K'_a value of $0.8 \times 1.08 \times 10^{-2} = 0.86 \times 10^{-2}$.

† The base, $[B]$, is calculated from the amount added in excess of the 100 cc. required to neutralize the first two hydrogens of H_3PO_4 .

Equation 35 is identical with Equation 17, which expresses the entire range of buffer values of a solution containing a weak acid plus amounts of strong acid or alkali not limited to equivalence with the weak buffer acid. It is evident therefore, that Equation 35, as an approximation, is valid in general for solutions containing acid buffers of all dissociation constants, at all pH's and concentration ranges, within which the mass action law holds—that is, for buffer concentrations up to about 0.1 M, and a pH range between 2 and 12, and perhaps as wide as between 1 and 13, and for amounts of added strong alkali or acid not limited to any relationship to the amount of buffer acid present.

It is evident from Equation 35 that when $[H^+]$ or $[OH']$ is of significant magnitude in comparison with $\frac{K'_a C}{(K'_a + [H^+])^2}$, the buffer effect will be correspondingly greater than that calculated from Equation 12 on the basis of Henderson's equation. Such is the case with the higher acidities attainable with $H_3PO_4 - BH_2PO_4$ mixtures, and with the higher alkalinities attainable with $HB_2PO_4 - B_3PO_4$ mixtures. Thus in Fig. 6 we have in the central part, the curve of $H(KHPO_4) - K(KHPO_4)$ mixtures, typical in shape and symmetry for a buffer acid acting in conformity with Henderson's equation, and with its center at $pH = pK'_a$; while the other two ends curve symmetrically towards horizontal asymptotes. The $H_3PO_4 - KH_2PO_4$ curve, however, instead of showing a decreasing slope as the proportion of free H_3PO_4 exceeds $\frac{1}{2}$ molecule, shows a slope constantly increasing to the end. This is because, as shown in Equation 35, the buffer effect of the $[H^+]$ itself, represented by the separate term $[H^+]$ in the equation $\beta = 2.3 \left(\frac{K'_a C [H^+]}{K'_a + [H^+]} + [H^+] + [OH'] \right)$, becomes so great, at the low pH (1.5) reached, that it prevents the decrease in the slope of the pH curve that otherwise occurs as $[Ha]$ approaches C , and in fact causes an increase. A similar increase of slope is observed, because of the high $[OH']$, at the alkaline end of the $HK_2PO_4 - K_3PO_4$ curve.

The quantitative rôles are indicated by Fig. 7 of the buffer effects due to incomplete dissociation of the phosphoric acids, and the buffer effects due to the $[H^+]$ and $[OH']$, respectively, at the

more extreme pH limits by the two lower curves in somewhat lighter lines than the upper curve representing the total effect. The curves are calculated by Equation 35 using K'_a values of 1.97, 6.85, and 12.0, respectively, for the three acids.

It will be noted that the β values at the alkaline end of the curve in Fig. 7 are somewhat less than indicated by the slope of this end of the curve in Fig. 6. The disagreement is not actual, however. Fig. 7 is calculated on the assumption of a constant volume with $C = 0.1$ M; while Fig. 6 represents the results of a titration in which the volume was greatly increased at the end. The disagreement would entirely disappear if the values of Fig. 7 were estimated on the basis of actual C and B values calculated from the varying volume.

Polyvalent Acid Buffers and Mixtures of Monovalent Acid Buffers.

For a solution containing a mixture of buffers, the buffer effect at any pH is the sum of the separate effects of each buffer in repressing $[H^+]$ change, plus the buffer effect peculiar to the $[H^+]$ and $[OH']$.

$$(36) \quad \beta = 2.3 [H^+] \left(\frac{K'_{a_1} C_1}{(K'_{a_1} + [H^+])^2} + \frac{K'_{a_2} C_2}{(K'_{a_2} + [H^+])^2} + \dots \right) + 2.3 ([H^+] + [OH'])$$

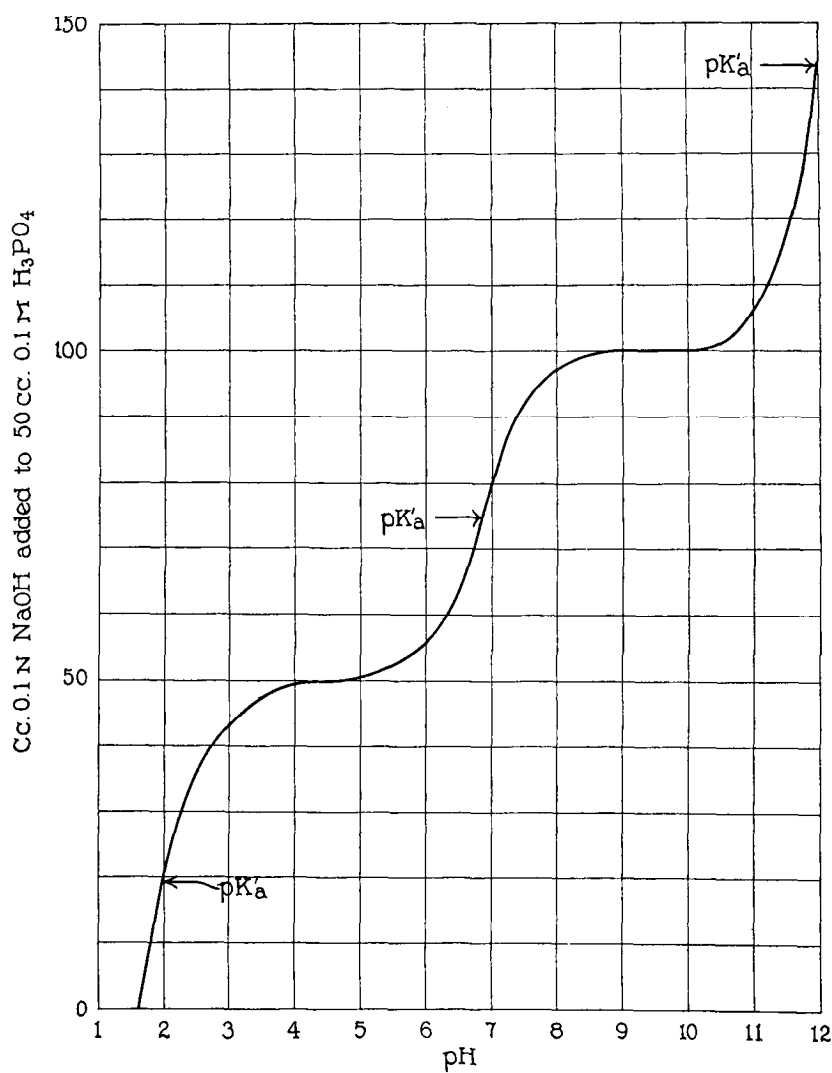
If $C_1 = C_2 = C_3$, as when the various acid groups belong to the same molecule of a polyvalent acid, Equation 36 simplifies to

$$(37) \quad \beta = 2.3 [H^+] C \left(\frac{K'_{a_1}}{(K'_{a_1} + [H^+])^2} + \frac{K'_{a_2}}{(K'_{a_2} + [H^+])^2} + \dots \right) + 2.3 ([H^+] + [OH'])$$

In a polyvalent buffer, or a mixture of buffers, the K'_a value and buffer effect β_1 , β_2 , or β_3 , etc., of any one acid or acid group may be determined, under the following conditions.

1. The pK'_a values of the different acids or acid groups are, as in H_3PO_4 , so far apart that the buffer effects do not overlap. In this case at $[H^+]$ values where β_1 is of significant magnitude, β_2 and β_3 are not, and $\beta = \beta_1$, β_2 , and β_3 being practically zero.

The extent to which a buffer exerts its effect on both sides of the point $pH = pK'_a$ is roughly indicated by the following table,

FIG. 6. Clark's titration curve of H_3PO_4 .

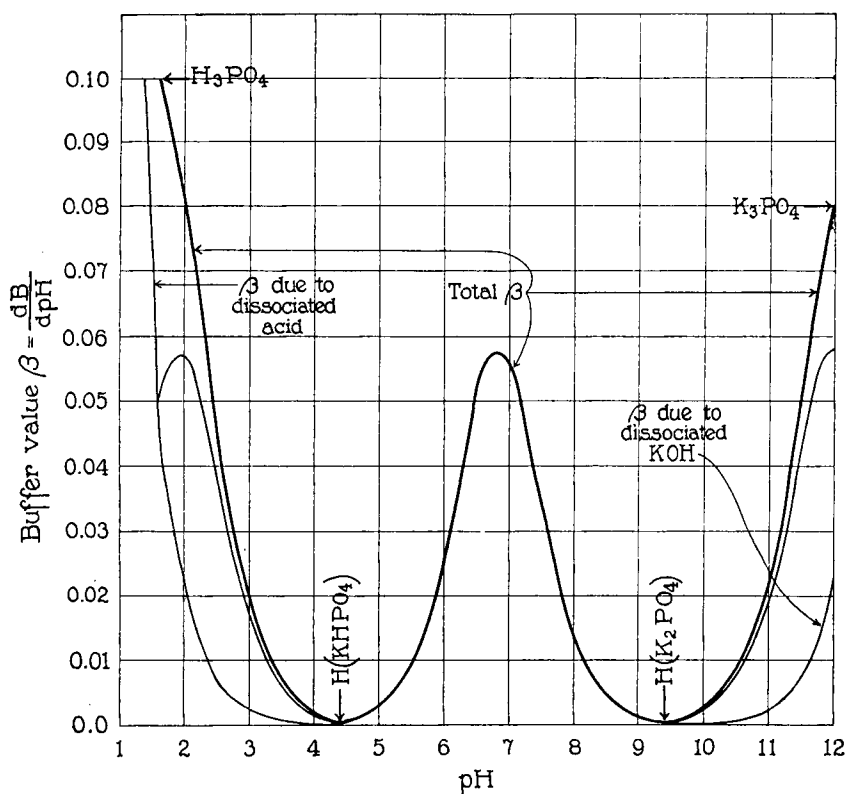


FIG. 7. Buffer values of the three phosphoric acids. The total buffer value is indicated by the heavy upper curve, which represents the total β value calculated by Equation 35.

At the more acid and alkaline ranges the partial β values due solely to dissociated free acid and alkali, respectively, become significant, and are indicated by the marked lower curves; while the remaining partial β values, due to repression of $[H^+]$ change, are indicated by symmetrical curves similar to that of $H(BHPO_4)$ in the center.

| pH | Proportion of maximal buffer effect. |
|---------------|--------------------------------------|
| | <i>per cent</i> |
| pK'_a | 100.0 |
| $pK'_a \pm 1$ | 33.0 |
| $pK'_a \pm 2$ | 4.0 |
| $pK'_a \pm 3$ | 0.4 |
| $pK'_a \pm 4$ | 0.04 |

(The effect is accurately shown for all values within the range $pH = pK'_a \pm 2$ by Fig. 9 in the attached note by Henderson.)

An example of a polyvalent buffer with acid groups whose buffer effects do not overlap is given, as stated above, by H_3PO_4 , and shown in Figs. 5 and 6.

2. *The buffer effects partially overlap.* In this case the calculations can be made from the parts of the curve where each buffer is free from the influence of the other, and can be checked by calculations of the total β and B in the pH range where they are summations of the overlapping effects.

3. *The effective range of a buffer is completely overlapped by those of others, of which, however, the K'_a and C values are known.* In this case we may assume for example that two buffers overlap with their buffer effects β_1 and β_2 the effect β_3 of a third buffer. If β_1 and β_2 can be calculated, β_3 can be estimated by subtracting β_1 and β_2 from the total observed buffer value,

$$\beta_3 = \beta - \beta_1 - \beta_2$$

From β_3 thus ascertained one may calculate K'_a by Equation 22. The calculation of β_1 and β_2 from known K'_{a_1} and K'_{a_2} , and of K'_{a_3} from β_3 , is made most simply, however, graphically, by inspection of the U-curve of Fig. 9.

When the total amount of base, B, is known, a similar procedure can be followed to use it instead of β to determine the dissociation constants. From Equation 33 one determines B_1 and B_2 , and by subtracting them from the total base B obtains B_3 . This is substituted in Equation 30 to determine K'_{a_3} . This procedure is somewhat simpler than that based on β determinations.

An example of the use of the above modes of procedure in determining the separate dissociation constants of a polyvalent acid is contained in a subsequent paper by Hastings and Van Slyke (1922) on citric acid.

It is to be noted that the maximum β_M of a polyvalent buffer may exceed 0.575 if the effects of the different buffer groups overlap. When $\beta = \beta_1 + \beta_2 + \beta_3$, if at the pH where β_2 has its maximum value of 0.575, β_1 and β_3 are of significant size, we shall have $\beta = 0.575 + \beta_1 + \beta_3$. In the case of citric acid, for example, the maximum β_M is 0.84. See also Fig. 8 for demonstration of the summation of buffer effects when the pK' values are nearer together than 3 units.

Basic Buffers.

If we designate the undissociated weak base as bOH (a small b is used to differentiate the weak base from B , the strong base), we have as our primary equation for dissociation of a weak base, Equation 38, analogous to Equation 5.

$$(38) \quad K_b = \frac{[OH'] [b^+]}{bOH}$$

If we have a mixture of a salt bA and the free base bOH , the equation becomes, analogous to Equation 28

$$(39) \quad K_b = \frac{[OH'] \left(\gamma_s A - \frac{\gamma_s}{\gamma_a} [H^+] + [OH'] \right)}{C - A + \frac{[H^+]}{\gamma_s} - [OH']}$$

If we let $K'_b = \frac{K_w \gamma_s}{K_b}$, substitute in Equation 39 $\frac{K_w \gamma_s}{K_b}$ for K_b , $\frac{K_w}{[H^+]}$ for $[OH']$, and $-B$ for A , and differentiate B with respect to $[H^+]$, as in obtaining Equation 35, we obtain an equation (Equation 40) identical with Equation 35, except that K'_b replaces K'_a .

$$(40) \quad \frac{dB}{dpH} = 2.3 \left(\frac{K'_b C [H^+]}{(K'_b + [H^+])^2} + [H^+] + [OH'] \right)$$

It follows, therefore, that all the equations and relationships that have been demonstrated to hold for acid buffers hold likewise for basic ones, the only difference being that in equations covering the behavior of the latter the approximate constant K'_b , which equals $\frac{K_w \gamma_s}{K_b}$, replaces the approximate constant K'_a which equals $\frac{K_a}{\gamma_s}$, of the equations covering the behavior of acid buffers.

Amphoteric Buffers.

In the case of an amphoteric buffer with acid groups, having K'_a values K'_{a_1} , K'_{a_2} , etc., and basic groups having K'_b values K'_{b_1} , K'_{b_2} , etc., we have, by summation

$$(41) \quad \beta = 2.3 C [H^+] \left(\frac{K'_{a_1}}{(K'_{a_1} + [H^+])^2} + \frac{K'_{a_2}}{(K'_{a_2} + [H^+])^2} \cdot \cdot \cdot + \frac{K'_{b_1}}{(K_w + K'_{b_1} [H^+])^2} + \frac{K'_{b_2}}{(K_w + K'_{b_2} [H^+])^2} \right) + 2.3 ([H^+] + [OH'])$$

The discussion under "Polyvalent acid buffers" applies also to amphoteric buffers, practically without modification except that pK'_b is to be used in place of pK'_a for the basic group.

Universal Buffer Mixtures.

Acree and his coworkers (1921) have pointed out that "there are advantages to be derived from the use of buffer mixtures covering a wide pH range . . . and so selected as to form a practically continuous, as well as smooth curve," in other words, to maintain a constant buffer value. From the considerations of the present paper it appears possible to predict the relationships of the buffers that will form such a mixture. Approximate constancy of buffer value will be maintained in a mixture of total buffer value

$$\beta = \beta_1 + \beta_2 + \beta_3 \cdot \cdot \cdot$$

under the following conditions. Each buffer must have its pK' at such a distance from its adjacent neighbor in the series that

the overlapping buffer effects at $\text{pH} = \text{pK}'_1$ equal those at $\text{pH} = \frac{\text{pK}'_1 + \text{pK}'_2}{2}$, or at a pH midway between two pK' values. Under these conditions the total buffer value midway between the maximum of a given buffer and that of the adjacent buffer will be the same as the buffer values at the maxima, and the fluctuations between can be but slight. The solution of the problem is approximated by calculating the total buffer values by means of Equation 12. The calculation is most readily done graphically by means of Fig. 9. Over most of the range covered in the calculations of Table II, it was necessary to add 5 buffer values at the point $\text{pH} = \text{pK}'_1$, and 6 at the point $\text{pH} = \frac{\text{pK}'_1 + \text{pK}'_2}{2}$, midway between 2 pK' 's.

TABLE IV.

| Interval between pK'_1 and $\text{pK}'_2 \dots \text{etc.}$ | Total buffer value, calculated as sum of molecular buffer values of individual buffers. | |
|---|---|---|
| | at $\text{pH} = \text{pK}'$ | at $\text{pH} = \text{pK}' + \frac{i}{2}$ |
| <i>i</i> | | |
| 3.0 | 0.577 | 0.138 |
| 2.0 | 0.598 | 0.384 |
| 1.6 | 0.684 | 0.552 |
| 1.4 | 0.749 | 0.673 |
| 1.3 | 0.784 | 0.738 |
| 1.2 | 0.848 | 0.813 |
| 1.1 | 0.919 | 0.899 |
| 1.0 | 1.003 | 0.998 |

It is apparent from Table IV that the ideal universal buffer would be approximated by a mixture of buffers forming a series with their pK' values separated by 1 unit, each K' being 10-fold as great as the next lower. In a solution containing such a series of buffers in equivalent concentration the total buffer value varies less than 1 per cent. When the intervals between pK' 's are 1.4, the total buffer value shows a difference of about 10 per cent between maxima and minima. When the difference is 2.0 the maximum buffer values exceed the minimum by over 50 per cent.

When the pK' intervals are regular, the most constant total buffer value is obtainable by using all buffers in the same concen-

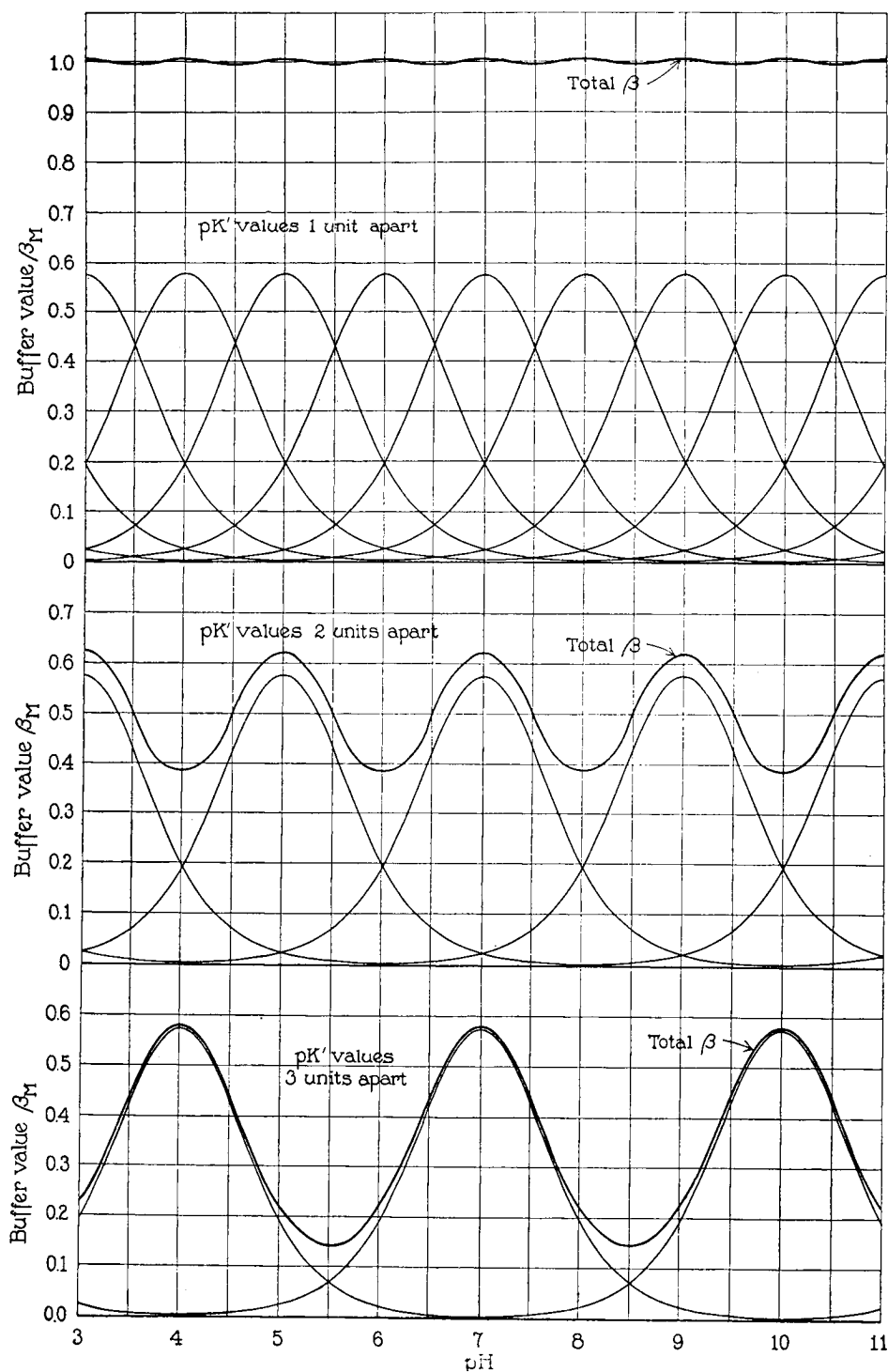


FIG. 8.

tration. In case, however, some of the intervals are longer than the rest so that one or two buffers are more isolated from the rest and consequently less supported by overlapping buffer effects of their neighbors, such buffers are to be used in sufficiently greater concentration to bring the total buffer value in these zones of activity up to that of the rest of the system.

Fig. 8 shows graphically the total buffer values obtained by equimolecular mixtures of buffer series, of which the pK' intervals are 1, 2, and 3, respectively.

The Buffer Value of Blood.

From the $BHCO_3$: pH curve for normal arterial blood under varying CO_2 tensions given by the writer in a previous article (1921, *b*, Fig. 2) it is possible to calculate the buffer value. At the reaction range pH 7.0 to 7.8, $dB = dBa$, since the amount of B in the form of BOH is negligible. The increase in $BHCO_3$ with increasing CO_2 tension and falling pH is equal to the decrease in Ba of the other buffers, since the base used to form additional $BHCO_3$ comes from them. Consequently, for the buffers

other than $BHCO_3$, $\frac{dB}{dpH} = \frac{-dBHCO_3}{dpH}$. Between pH 7.0 and

7.8 (mean pH = 7.4; $\Delta pH = 0.8$) the mean molecular concentration of $BHCO_3$ decreases from 0.0285 to 0.0120 ($\Delta Ba = 0.0165$). Therefore, at mean pH 7.4, for the buffers other than $BHCO_3$,

$$\beta = \frac{\Delta Ba}{\Delta pH} = \frac{0.0165}{0.8} = 0.0206.$$

The buffers other than $BHCO_3$ are chiefly proteins. In fact, they appear to be chiefly the weak acid groups in the hemoglobin (Van Slyke, 1921, *a*).⁶ If these groups were exerting their maximum buffer efficiency, having their pK' values near 7.4, their

total concentration would be $C = \frac{\beta}{0.575} = \frac{0.0206}{0.575} = 0.036$ N

(Equation 20), and they would be combined with 0.5 equivalent of alkali, or 0.018 N, since, at $pH = pK'$, $Ba = \frac{C}{2}$. If pK' were

less than 7.4, then Ba would exceed 0.018 N, if pK' were more than 7.4, then Ba, the base bound to protein, would be less than 0.018 N.

⁶ Van Slyke (1921, *a*), p. 160.

As a matter of fact, as closely as one can estimate from the only data available (Greenwald, 1922), 0.018 *N* is about the concentration of alkali bound to proteins in the blood. If this is substantiated (experiments are in progress), it means that in normal blood the active buffer groups, other than those of BHCO_3 , total about 0.036 *N* in concentration. The hemoglobin concentration is about 0.008 *M*, if one assumes that 1 molecule of hemoglobin binds 1 molecule of oxygen. Probably, therefore, $\frac{3.6}{0.8} = 4.5$ represents more or less approximately the number of COOH groups in each oxyhemoglobin molecule that are active at blood pH.

In Greenwald's method the proteins are precipitated with an excess of picric acid and the picric acid combined with alkali is determined as the difference between total picrate and free titratable picric acid. Essentially this procedure removes the protein and total H_2CO_3 , and measures the picric acid required to neutralize the bases that were combined with them. In order to measure this value exactly in such a procedure the titration of the free picric acid would require the pH of blood for its end-point (Van Slyke, Stillman, and Cullen, 1919).⁷ Greenwald used two end-points, one (methyl red) 2 pH units on the acid side of 7.4, the other (thymolphthalein) about equally on the alkaline side. The average normal concentrations of base thus measured were 0.0435 and 0.0315 *N*, respectively, with the two indicators, the mean value, 0.0375 *N* being probably close to the amount of base bound by H_2CO_3 and protein in normal blood. If we subtract from 0.0375 *N* the 0.0205 *N* base normally bound as bicarbonate (Van Slyke, 1921, *b*),⁸ we have left 0.0170 *N* base bound by the proteins.

If to the buffer value of the buffers other than the bicarbonate we add that of bicarbonate we obtain the total buffer value of the blood. The value for the BHCO_3 and H_2CO_3 buffer at pH 7.4 may be calculated from Equation 12. At pH 7.4, $[\text{H}^+] = 4 \times 10^{-8}$; $C = [\text{BHCO}_3] + [\text{H}_2\text{CO}_3] = 0.0215$; and $K_a' = 8 \times 10^{-7}$ (Henderson and Haggard). Substituting these numerical values in Equation 12 we have

$$\frac{dB}{dpH} = 2.3 \frac{4 \times 10^{-8} \times 0.0215 \times 8 \times 10^{-7}}{(8 \times 10^{-7} + 4 \times 10^{-8})^2} = 0.0022$$

Adding 0.0022 to 0.0206, the β of the other buffers, gives $\beta = 0.0228$ as the average total buffer value of normal human blood at pH 7.4.

⁷ Van Slyke, Stillman, and Cullen (1919), p. 167.

⁸ Van Slyke (1921, *b*), p. 170.

The total *concentration* of buffers active in the blood at pH 7.4 is at least 0.036 N (buffers other than bicarbonate) + 0.021 N ($\text{BHCO}_3 + \text{H}_2\text{CO}_3$) = 0.057 N. The alkali bound by these buffers, which are apparently all of the weak acid type, appears to be about 0.04 N (0.038 N bound by protein and H_2CO_3 (Greenwald, 1922), and traces by other buffers).

The above estimates of buffer concentration and buffer alkali are merely first approximations. The 0.04 N buffer alkali figure is a rough estimate. The 0.057 N total buffer concentration may have to be increased in proportion as the active protein buffer groups are found to have pK' values differing from 7.4. The figure 0.022 for the average $\frac{dB}{dpH}$ value of human blood is based on fairly complete observations and is probably about correct.

The ability of the blood *in vivo* to neutralize *non-volatile acids without change in pH* is not due to buffer action, that is to the incomplete dissociation of the weak buffer acids set free by reactions such as $\text{HCl} + \text{Ba} = \text{Ha} + \text{BCl}$. It is due to the volatility of one of these acids, CO_2 , and the ability of the respiratory apparatus to remove the excess CO_2 set free by such a reaction as $\text{HCl} + \text{BHCO}_3 = \text{BCl} + \text{H}_2\text{CO}_3$. Because CO_2 set free is thus removed, the bicarbonate is, as shown by the writer (1921, *a*), the most important form of the blood's alkali reserve available for neutralizing non-volatile acids, such as β -hydroxybutyric.

For neutralization of added H_2CO_3 , however, (in venous blood) the BHCO_3 formed can exert only its actual buffer effect, which is about 0.1 that of the other blood buffers. For preservation of the blood's neutrality against retention of non-volatile acids the bicarbonate, because of the volatility of the CO_2 set free from it, is the most important single constituent; while for preservation of neutrality against CO_2 retention, hemoglobin, because of its buffer power, is the most important constituent.

Determination of the Buffer Values of a Solution by Titration.

The experimental determination of the buffer value, β , of a given solution at a given pH may be performed in either of the following two ways.

1. *By determination of the slope of the B, pH curve at the given pH.* One adds strong standard acid or alkali in varying amounts

to the solution, and determines the pH after each addition. If the concentration of added standard acid or alkali be great enough to keep the maximum increase in volume of the buffer solution relatively small, *e.g.* below 50 per cent of the original, the volume change may ordinarily be neglected, as the pH values of most buffer solutions are but slightly affected by volume changes of such magnitude. Addition of each cc. of *N* NaOH to a buffer solution of original volume *V* cc. causes an increase of $\Delta B = \frac{1}{V}$.

The buffer value, $\frac{dB}{dpH}$, at any pH may be determined graphically by drawing a line tangent to the curve at that pH and measuring the slope of the line. (See Fig. 1 and its discussion.) (The tangent can be drawn most accurately by a device employed by engineers and called to my attention by Dr. Northrop. A mirror is laid at right angles across the curve, so that the curve on paper is continuous with its reflection. A line drawn across the curve along the edge of the mirror is then perpendicular to the tangent, which with the help of a square is laid off at right angles to the mark of the mirror-edge.)

2. *By measuring the amount of strong acid or alkali required to cause a pH change over a measured range.* One merely adds as in the above procedure, a measured amount of standard alkali or acid, and determines the pH before and after the addition. If the added alkali is ΔB , and the increase in pH is ΔpH , then the average buffer value between the two pH's observed is $\beta = \frac{\Delta B}{\Delta pH}$.

For example, if 10 cc. of *M* NaOH are added to 1 liter of a buffer solution of unknown value, $\Delta B = \frac{10 \times 1}{1,000} = 0.01$. If the pH values before and after the additions are 7 and 8.5, respectively $\Delta pH = 8.5 - 7 = 1.5$, $\beta = \frac{0.01}{1.5} = 0.0067$. 0.0067 represents the average value of β between pH 7 and 8.5, or approximately the β at pH 7.75. Table I gives an example of this manner of determining values of β .

If the average β value over a certain pH range is used as an approximation of the exact β value at the mean pH, as in Table

I, it is essential that the pH intervals be not too great. Where the B, pH curve is nearly straight a pH range exceeding 1 may be used to determine β at the mean pH, as in the case of citric acid; but where there is much curvature the range should be less in order to yield accurate mean values.

TABLE V.

| $\frac{[\text{Ba}]}{[\text{C}]} \text{ or } \frac{\text{bOH}}{\text{C}}$ | $\frac{[\text{Hcl}]}{\text{C}} \text{ or } \frac{\text{bA}}{\text{C}}$ | $\frac{[\text{H}^+]}{\text{K}}$ | $\log \frac{[\text{H}^+]}{\text{K}} = \text{pK}' - \text{pH}$ | $0.4343 \beta_M = V$ | $\frac{dV}{d \log [\text{H}^+]}$ | $\frac{\beta}{0.575} = U$ |
|--|--|---------------------------------|---|----------------------|----------------------------------|---------------------------|
| 0.01 | 0.99 | 99.0 | 1.996 | 0.0099 | -0.0097 | 0.0396 |
| 0.02 | 0.98 | 49.0 | 1.690 | 0.0196 | -0.0192 | 0.0784 |
| 0.05 | 0.95 | 19.0 | 1.279 | 0.0475 | -0.0428 | 0.1900 |
| 0.10 | 0.90 | 9.0 | 0.954 | 0.0900 | -0.0720 | 0.3600 |
| 0.15 | 0.85 | 5.67 | 0.753 | 0.1275 | -0.0893 | 0.5100 |
| 0.20 | 0.80 | 4.00 | 0.602 | 0.1600 | -0.0960 | 0.6400 |
| 0.25 | 0.75 | 3.00 | 0.477 | 0.1875 | -0.0938 | 0.7500 |
| 0.30 | 0.70 | 2.33 | 0.368 | 0.2100 | -0.0840 | 0.8400 |
| 0.35 | 0.65 | 1.858 | 0.269 | 0.2275 | -0.0683 | 0.9100 |
| 0.40 | 0.60 | 1.500 | 0.176 | 0.2400 | -0.0480 | 0.9600 |
| 0.45 | 0.55 | 1.222 | 0.087 | 0.2475 | -0.0248 | 0.9900 |
| 0.50 | 0.50 | 1.000 | 0.000 | 0.2500 | 0.0000 | 1.0000 |
| 0.55 | 0.45 | 0.818 | 0.913-1 | 0.2475 | 0.0248 | 0.9900 |
| 0.60 | 0.40 | 0.667 | 0.824-1 | 0.2400 | 0.0480 | 0.9600 |
| 0.65 | 0.35 | 0.538 | 0.731-1 | 0.2275 | 0.0683 | 0.9100 |
| 0.70 | 0.30 | 0.429 | 0.632-1 | 0.2100 | 0.0840 | 0.8400 |
| 0.75 | 0.25 | 0.333 | 0.523-1 | 0.1875 | 0.0938 | 0.7500 |
| 0.80 | 0.20 | 0.250 | 0.398-1 | 0.1600 | 0.0960 | 0.6400 |
| 0.85 | 0.15 | 0.176 | 0.247-1 | 0.1275 | 0.0893 | 0.5100 |
| 0.90 | 0.10 | 0.111 | 0.046-1 | 0.0900 | 0.0720 | 0.3600 |
| 0.95 | 0.05 | 0.0526 | 0.721-2 | 0.0475 | 0.0428 | 0.1900 |
| 0.98 | 0.02 | 0.0204 | 0.310-2 | 0.0196 | 0.0192 | 0.0784 |
| 0.99 | 0.01 | 0.0101 | 0.004-2 | 0.0099 | 0.0097 | 0.0396 |

50 per cent } 75 per cent

Note by Lawrence J. Henderson.

Professor Henderson consented to criticize the preliminary draft of the above paper and suggested that the relationships involved in the first and second differential derivatives of his equation (Equation 7) might be more readily perceived as out-

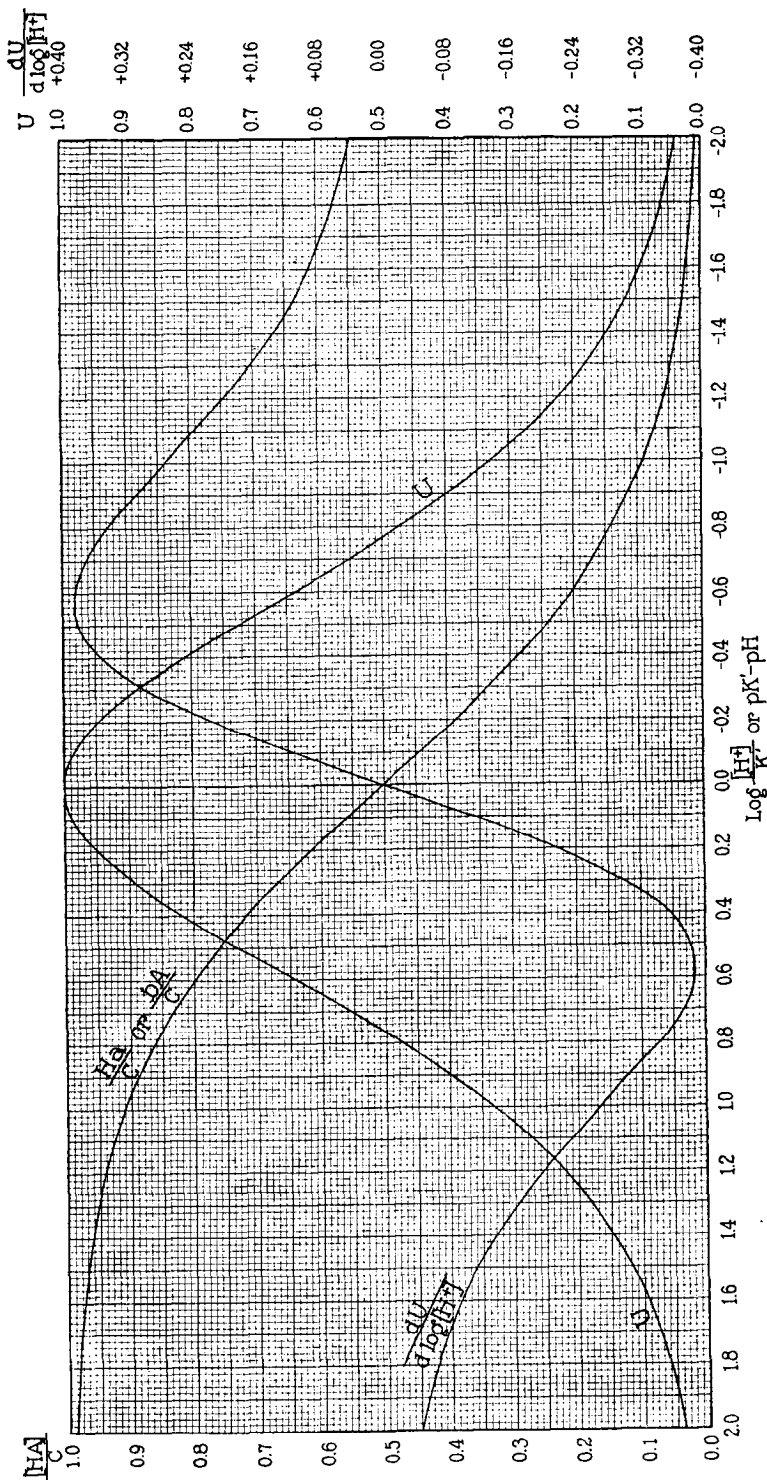


FIG. 9.

lined by the following series of equations, and by Table V and Fig. 9. The latter demonstrate over the main significant range, the interrelation between: (1) buffer effect, (2) ratio of buffer dissociation constant to hydron concentration of the solution, (3) ratio of buffer salt to free buffer base or acid, and (4) value of the second differential.

First Derivative.

Given a buffer mixture such that

$$[\text{Ha}] + [\text{Ba}] = C$$

If
$$[\text{H}^+] = K \frac{[\text{Ha}]}{[\text{Ba}]}$$

$$[\text{H}^+] = K \frac{[\text{Ha}]}{C - [\text{Ha}]}$$

$$[\text{H}^+] C = [\text{H}^+] [\text{Ha}] + K [\text{Ha}]$$

$$[\text{Ha}] = \frac{[\text{H}^+] C}{K + [\text{H}^+]}$$

Imagine addition of strong acid or strong base

$$\frac{d[\text{Ha}]}{d[\text{H}^+]} = \frac{(K + [\text{H}^+]) C - [\text{H}^+] C}{(K + [\text{H}^+])^2} = \frac{C}{K + [\text{H}^+]} - \frac{C}{K + [\text{H}^+]} \times \frac{[\text{H}^+]}{K + [\text{H}^+]}$$

$$\frac{d[\text{Ha}]}{d[\text{H}^+]} = \frac{C}{K + [\text{H}^+]} \left(1 - \frac{[\text{H}^+]}{K + [\text{H}^+]} \right) = \frac{C}{K + [\text{H}^+]} \times \frac{K}{K + [\text{H}^+]}$$

$$\beta_M \times \log_e 10 = V = \frac{d[\text{Ha}]}{d[\text{H}^+]} \times \frac{d[\text{H}^+]}{d \log [\text{H}^+]} \times \frac{1}{C} = \frac{d[\text{Ha}]}{d \log [\text{H}^+]} \times \frac{1}{C} = \frac{[\text{H}^+]}{K + [\text{H}^+]} \times \frac{K}{K + [\text{H}^+]}$$

$$V = \frac{1}{1 + \frac{[\text{H}^+]}{K}} \times \frac{1}{1 + \frac{K}{[\text{H}^+]}} \dots \dots \dots (1)$$

But
$$\frac{[\text{Ha}]}{C} = \frac{[\text{H}^+]}{K + [\text{H}^+]} = \frac{1}{1 + \frac{K}{[\text{H}^+]}}$$

And
$$\frac{[\text{Ba}]}{C} = \frac{1}{1 + \frac{[\text{H}^+]}{K}}$$

Hence alternatively, leaving out the constant factor $\frac{1}{\log_e 10}$,

$$\begin{cases} \beta_M = \frac{[\text{Ha}]}{C} \times \frac{[\text{Ba}]}{C} \\ \beta_M = \frac{[\text{Ha}]}{C} - \frac{[\text{Ha}]^2}{C^2} \\ \beta_M = \frac{[\text{Ba}]}{C} - \frac{[\text{Ba}]^2}{C^2} \end{cases}$$

Second Derivative.

$$\begin{aligned}
\beta_M \times \log_e 10 = V &= \frac{H}{K + [H^+]} \times \frac{K}{K + [H^+]} \\
\frac{dV}{d[H^+]} &= \frac{K}{K + [H^+]} \times \frac{(K + [H^+]) - [H^+]}{(K + [H^+])^2} + \frac{[H^+]}{K + [H^+]} \times \frac{-K}{(K + [H^+])^2} \\
\frac{dV}{d \log [H^+]} &= \frac{dV}{d[H^+]} \times \frac{d[H^+]}{d \log [H^+]} = \frac{K}{K + [H^+]} \times \frac{K}{K + [H^+]} \times \frac{[H^+]}{K + [H^+]} - \\
&\quad \frac{K}{K + [H^+]} \times \frac{[H^+]}{K + [H^+]} \times \frac{[H^+]}{K + [H^+]} \\
\frac{dV}{d \log [H^+]} &= V \left(\frac{1}{1 + \frac{[H^+]}{K}} - \frac{1}{1 + \frac{K}{[H^+]}} \right) \dots \dots \dots (2)
\end{aligned}$$

Hence, alternatively, leaving out the constant factor $\frac{1}{(\log_e 10)^2}$

$$\frac{dV}{d \log [H^+]} = \frac{[Ha]}{C} \times \frac{[Ba]}{C} \times \left(\frac{[Ba]}{C} - \frac{[Ha]}{C} \right)$$

SUMMARY.

As a numerical measure of the buffer value of a solution the number of gram equivalents of strong alkali or acid taken up per unit change in pH has been used. In these terms, each cc. of *N* alkali or acid that must be added to a liter of solution to raise or lower its pH by 1 adds 0.001 to the buffer value.

Since the buffer value varies with varying pH, the value at any given pH is defined by the ratio $\frac{dB}{dpH}$.

A general form of the mass action equations, $K = \frac{[H^+][a']}{[Ha]}$

and $K = \frac{[OH'] [b']}{[bOH]}$, covering the dissociations of weak acids and bases, respectively, in the presence of their salts, has been developed and differentiated. The general equation obtained expressing the buffer value, β , is

$$\beta = \frac{dB}{dpH} = 2.3 \left(\frac{K' [H^+] C}{(K' + [H^+])^2} + [H^+] + [OH'] \right)$$

$K' = \frac{K_a}{\gamma_s}$ for weak acids, where K_a is the acid dissociation con-

stant, γ_s , the fraction of the salt Ba dissociated into $[B^+]$ and $[a']$ ions. For weak bases $K' = \frac{K_w \gamma_s}{K_b}$. C indicates the concentration of the buffer. When $C = 0$, and no weak acid or base is present, the buffer equation simplifies to $\beta = 2.3 ([H^+] + [OH'])$, which indicates the buffer value of water plus strong acid or alkali.

When pH is within the limits of about 3 and 11, and C has a value not much less than 0.1 N, $[H^+]$ and $[OH']$ are relatively so small that the buffer equation simplifies to

$$\beta = 2.3 \frac{K' [H^+] C}{(K' + [H^+])^2}$$

Under these conditions the molecular buffer value $\beta_M = \frac{\beta}{C} = 2.3 \frac{K' [H^+]}{(K' + [H^+])^2}$. β has its maximum value for every buffer when $[H^+] = K'$. At this point $\beta_M = \frac{2.3}{4} = 0.575$ for every buffer, and one-half the buffer is in the form of free acid or base, one-half in the form of its salt. That is, maximum buffer value is exerted when $\frac{[H^+]}{K'} = \frac{Ba}{Ha} \left(\text{or} = \frac{bOH}{bA} \right) = 1$. For given values of these ratios greater or less than 1 definite proportions of the maximum buffer effect are exerted (Fig. 9).

Applications of the above facts are indicated, including the determination of the acid and basic dissociation constants and the molecular weights of buffers, and the calculation of the buffer values of solutions of mixed, polyvalent, and amphoteric buffers. The technique for experimental determination of the buffer values of solutions is outlined.

The buffer value of normal blood at pH 7.4 is shown to approximate that of a 0.04 N solution of a buffer acid with $K' = 10^{-7.4}$

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Note Added to Proof.—Koppel and Spiro (1914), in a paper overlooked until the above went to press, have utilized a similar, though not identical buffer unit, viz. $\frac{d(S-S_0)}{dpH}$, where S_0 represents the amount of strong acid that would produce the change dpH if added to an unbuffered solution of a given initial pH, while S represents the actual amount of strong acid required in the buffered solution to cause the same pH change. Within the pH range of validity of Henderson's equation this unit becomes numerically identical with ours (except for being its negative value), since S_0 is negligible; and Koppel and Spiro have reached some conclusions identical with ours for solutions within this range, viz., concerning the occurrence of a constant, maximum, buffer value when $K_a=[H^+]$. For pH values outside the range of validity of Henderson's equation, Koppel and Spiro's unit is not identical with the, we believe, more simply applied $\frac{dB}{dpH}$ unit developed in this paper.

**ON THE MEASUREMENT OF BUFFER
VALUES AND ON THE RELATIONSHIP
OF BUFFER VALUE TO THE
DISSOCIATION CONSTANT OF THE
BUFFER AND THE CONCENTRATION
AND REACTION OF THE BUFFER
SOLUTION**

Donald D. Van Slyke

J. Biol. Chem. 1922, 52:525-570.

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