The buffer power of blood: a reappraisal of its mathematical expressions with implications on the role of albumin as a buffer

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The buffer power (β) of a solution describes its capacity of limiting pH changes induced by the addition of acid or base (1). When discussing the addition of acids, two main formulations exist to define β: d[dissociated acid]/dpH, as introduced by Donald Van Slyke (1), and [added acid]/d[H^+], as proposed by Peter Stewart (2). The present work describes merits and pitfalls of these two approaches, highlighting clinical implications with particular reference to the role of albumin as a buffer in blood. The stimulus for this viewpoint stems from a recent publication by Wolf suggesting that albumin "compromises" buffering, as it *reduces* blood's β when calculated with Stewart's formulation (3). Although supported by previous theoretical investigations (2, 4, 5), such conclusion is in contrast with experimental data showing that albumin *increases* blood's β when computed with Van Slyke's formulation (6). We therefore think that this controversy might benefit from further clarification.

Abbreviations

A: [added acid];
H^+: [added hydrogen ions];
A^-: [added dissociated acid];
[A]: [undissociated acid in solution];
[A^-]: [dissociated acid in solution];
[H^+]: [hydrogen ions in solution];
β_{VS}: Van Slyke's β = d[A^-]/dpH;
β_{PS}: Stewart's β = A/d[H^+];
K: dissociation constant;
pK: negative logarithm of K;
PCO_2: partial pressure of carbon dioxide;
[HCO_3^-]: [bicarbonate ion];
SID: [strong ion difference];
[Alb]: [albumin].

Van Slyke's approach

Donald Van Slyke initially defined β as the amount of A required to obtain a unitary change in pH: $\beta = A/dpH$. When a *strong acid* is added to a solution, it completely dissociates into A^- and H^+, thereby $\beta = A/dpH = H^+/dpH$. Conversely, when a *weak acid* is added, part of it remains in solution as [A], part dissociates into equal amounts of A^- and H^+, thereby $\beta = A/dpH$ differs from H^+/dpH. Since in both cases (*strong* or *weak* acid titration) H^+ equals A^-, and the latter equals $d[A^-]$, Van Slyke proposed the expression $d[A^-]/dpH$ (β_{VS}) to obtain the same buffer value irrespective of the type of titration (1). Accordingly, experiments using solutions containing only albumin and strong ions have confirmed that the same β_{VS} is obtained by titration with the *strong* hydrochloric acid or with the *weak* acid CO_2: $\beta_{VS} = d[Cl^-]/dpH = d[HCO_3^-]/dpH$ (7, 8). Of note, CO_2 is not a weak acid per se, but it behaves as such in blood (apparent pK = 6.1) (9).

As an alternative to experimental titration, in the clinical pH range β_{VS} can be calculated as:

Equation 1

$$\beta_{VS} = 2.303 \cdot c \cdot \frac{(10^{-(pK+pH)})}{(10^{-pK} + 10^{-pH})^2}$$

Where c is the concentration of buffers in solution, and pK is their negative logarithmic dissociation constant (1).

Equation 1 encompasses the two main properties of β_{VS}, both of which have been experimentally confirmed:

1) it is *zero* in the absence of buffers (Figure, Panel B), and increases linearly with their concentration "c" (Figure, Panel A) (6);
2) it increases as pH approaches the pK of buffers in solution (10). Accordingly, since blood buffers' pK is lower than 7.4 (11), β_{VS} increases as blood becomes acidic (Figure, Panel A).

Stewart's approach

In the late 70's, Peter Stewart proposed the alternative formulation $\beta_{PS} = A/d[H^+]$, changing both the numerator and denominator of β_{VS} (2). His arguments were as follows:

1. *Numerator*: the variable that determines the variation in $[H^+]$ is A, while $[A^-]$ depends on several other factors (12). Despite the sound physical-chemical rational, this approach creates a problem when titration with *strong* and *weak* acids is compared. For instance, SID and PCO_2 are both independent variables in blood, but the former behaves like a *strong* acid, the latter like a *weak* acid. Consequently, for the same A, H^+ is lower during titration with PCO_2, leading to a higher β_{PS} when compared to titration with SID (2). This is the *first discrepancy* with β_{VS}, which is instead lower during PCO_2 titration: indeed, only non-carbonic species act like buffers in this case, whereas during titration with SID, also bicarbonate participates to buffering, increasing β_{VS} (11). Although it might be of some clinical interest to remember that blood is less protected against SID than PCO_2 variations (as β_{PS} shows), this is simply due to SID behaving like a *strong* acid, and not to the buffering properties of blood (described instead by β_{VS}).
2. *Denominator*: The use of $[H^+]$ unravels an important buffering property of solutions titrated with *weak* acids, which is not evident when using pH (2). Imagine a water solution with PCO_2 and strong electrolytes, where SID = $[HCO_3^-]$ (both in mMol/L), $[H^+]$ is in nMol/L, and non-carbonic buffers are absent. Titration of such solution with PCO_2 (our clinical surrogate of *weak* acids) obeys the Henderson equation (13):

   Equation 2

$$[H^+] = K \cdot \frac{PCO_2}{[HCO_3^-]}$$

   At varying PCO_2, $[HCO_3^-]$ can be considered constant since, in the absence of non-carbonic buffers, its increase is in the order of nMol/L, *i.e.*, insignificant with respect to its initial

concentration in mMol/L. Accordingly, the fractional change in PCO_2 equals the fractional change in $[H^+]$ (*e.g.*, if the former doubles, the latter doubles). Consequently, the same absolute change in PCO_2 causes a different absolute change in $[H^+]$ depending on the initial value of $[H^+]$ in solution. In other words, during CO_2 titration, the more acidic the initial solution, the lower the β_{PS} calculated as $dPCO_2/d[H^+]$ (Figure, Panel B). In the presence of non-carbonic buffers, the relationship between β_{PS} and the initial $[H^+]$ of the solution has a lower slope due to the concomitant increase in $[HCO_3^-]$, but the inverse proportionality remains (Figure, Panel A). This is the *second*, and *most important discrepancy* with β_{VS}, whereby such property is mathematically masked by the transformation of $[H^+]$ into its logarithmic expression, *i.e.* pH (see also $\beta_{PS(pH)} = dPCO_2/dpH$ in the Figure, Panels A-B) (2).

Of note, Wolf correctly pointed out that β_{PS} should be written as $d[H^+]/A$, being A the independent variable (3). Here, we decided to use its inverse ($\beta_{PS} = A/d[H^+]$) for an easier comparison with β_{VS}, where the hydrogen component is at the denominator ($\beta_{VS} = d[A^-]/dpH$).

Clinical implications

The physiological controversy between β_{VS} and β_{PS} finds its clinical implication in the role of albumin as a buffer. While β_{VS} increases with [Alb] (1, 6, 11), Stewart and others have shown that β_{PS} decreases when [Alb] increases (2–4), concluding that albumin is not a buffer. This paradox can be solved by considering that, together with its pH-dependent positive charges (imidazole groups) behaving as buffers, albumin has a much larger component of fixed negative charges behaving as *strong* acids (14). Accordingly, when albumin increases in blood, pH decreases (as demonstrated *in-vitro*) (15). Now, if one performs PCO_2 titration at different [Alb], β_{VS} increases with [Alb] because "c" in Equation 1 increases (6), and the solution's pH decreases, approaching the imidazoles' pK (16). Conversely, β_{PS} decreases with increasing [Alb], because the initial $[H^+]$ of the solution

increases (3, 4) (Figure, Panel A). Such negative relationship between β_{PS} and [Alb] is thereby a consequence of the acidifying effect of albumin's fixed charges and it is not related to its buffering role due to the imidazole groups. Indeed, a similar decrease in β_{PS} would be observed if the initial $[H^+]$ of the solution was altered by SID, irrespective of [Alb] (Figure, Panel B). Moreover, when corrected for the effect of the initial $[H^+]$, β_{PS} actually increases with [Alb] (Figure Legend). Interestingly, the latter scenario better reflects *in-vivo* data, since clinical variations in [Alb] are frequently not associated with the expected *in-vitro* changes in pH (17, 18), as the body compensates by changing chloride concentration and/or PCO_2 (19, 20). Accordingly, patients with higher [Alb] have a higher β (calculated both as β_{VS} and β_{PS}), confirming that albumin acts as a buffer in blood (6) (Figure, Panels C-D).

Conclusions

Van Slyke's β links the concept of buffer power to the presence, concentration and pK of blood buffers; it is independent of the acid used for titration (*strong* or *weak*), and its mathematical equation is consistent with experimental and clinical data. Conversely, β_{PS} changes with the strength of the acid used for titration; it depends on the initial $[H^+]$ of the solution (regardless of the presence of buffer species) and only adds information on the protection against changes in $[H^+]$ which are not necessarily paralleled by changes in pH. When thoroughly analyzed, β_{PS} and β_{VS} are concordant in showing that albumin acts as a buffer in blood, but the former does so less intuitively. We therefore favor the use of β_{VS} for the clinical description of blood's buffering properties.

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Figure legend

Panel A: a reproduction of Figure 1 from Wolf (3): CO_2 titration of blood at different [Alb]. A strong relationship is found between [Alb] and the initial $[H^+]$ of the solution, *i.e.*, $[H^+]$ at PCO_2 20 mmHg ($[H^+]_{20}$): $[H^+]_{20} = 4.174 \cdot [Alb] + 6.510$ ($r = 0.99$, $p<0.01$). Since, from Equation 2, $[H^+]_{20}$ correlates negatively with β_{PS} ($\beta_{PS} = -0.018 \cdot [H^+]_{20} + 1.989$ ($r = 0.98$, $p<0.01$)), an inverse proportionality exists between β_{PS} and [Alb] ($\beta_{PS} = -0.074 \cdot [Alb] + 1.867$ ($r = 0.96$, $p<0.01$), possibly suggesting that albumin is not a buffer. However, when correcting for the effect of $[H^+]_{20}$ in a multilinear regression, β_{PS} actually increases with [Alb] ($\beta_{PS} = 0.072 \cdot [Alb] - 0.035 \cdot [H^+]_{20} + 2.093$ ($r = 0.99$, $p<0.01$)). Moreover, the relationship between β_{PS} and [Alb] becomes positive when pH is used as denominator (see $\beta_{PS(pH)}$), in accordance with the expected trend in β_{VS}: $\beta_{VS} = 1.567 \cdot [Alb] + 12.932$ ($r = 0.74$, $p<0.01$). **Panel B**: theoretical CO_2 titration of solutions having the same $[H^+]_{20}$ as those in Panel A, and SID equal to the corresponding $[HCO_3^-]$ at PCO_2 20 mmHg, as calculated from Equation 2 ($[HCO_3^-]$ considered constant in the absence of non-carbonic buffers). As shown, β_{VS} is *zero* since no buffers are present (as expected from Equation 1). Additionally, the inverse proportionality between $[H^+]_{20}$ and β_{PS} remains ($\beta_{PS} = -0.025 \cdot [H^+]_{20} + 1.525$ ($r = 0.95$, $p<0.01$)), confirming its independence from the presence of buffers in solution. However, the relationship is lost when pH is used as denominator (see $\beta_{PS(pH)}$). Moreover, the slope $d\beta_{PS}/d[H^+]_{20}$ is more negative than in Panel A (-0.025 ± 0.0006 *vs.* -0.018 ± 0.0003, Student's $p<0.01$) suggesting that buffers mitigate the effect of $[H^+]_{20}$ on β_{PS}. Finally, at the same $[H^+]_{20}$, the difference between β_{PS} in Panels A and B increases with [Alb], further highlighting its buffering role. This is confirmed by the experimental data from our group (6) in **Panel C**, comparing CO_2 titration in plasma (no hemoglobin) of 18 healthy subjects and 18 septic patients: both β_{PS} and β_{VS} are lower in septic, hypoalbuminemic patients. Indeed, the relationship between [Alb] and $[H^+]_{20}$ in the overall population is not positive, but rather slightly negative: $[H^+]_{20} = -2.766 \cdot [Alb] + 30.78$ ($r = 0.48$, $p<0.01$)). Accordingly, while the inverse proportionality between β_{PS} and $[H^+]_{20}$ remains ($\beta_{PS} =$ -

237 $0.038 \cdot [H^+]_{20} + 1.882$ ($r = 0.89$, $p<0.01$)), the relationship between β_{PS} and [Alb] is positive ($\beta_{PS} =$

238 $0.159 \cdot [Alb] + 0.509$ ($r = 0.66$, $p<0.01$), like the one between β_{VS} and [Alb] ($\beta_{VS} = 1.035 \cdot [Alb] -$

239 1.068 ($r = 0.82$, $p<0.01$)), confirming that albumin acts as a buffer (**Panel D**).

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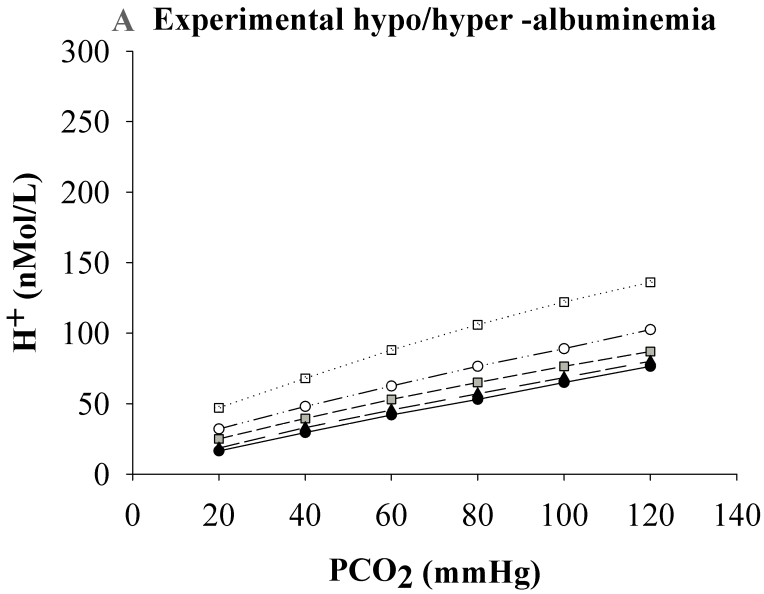
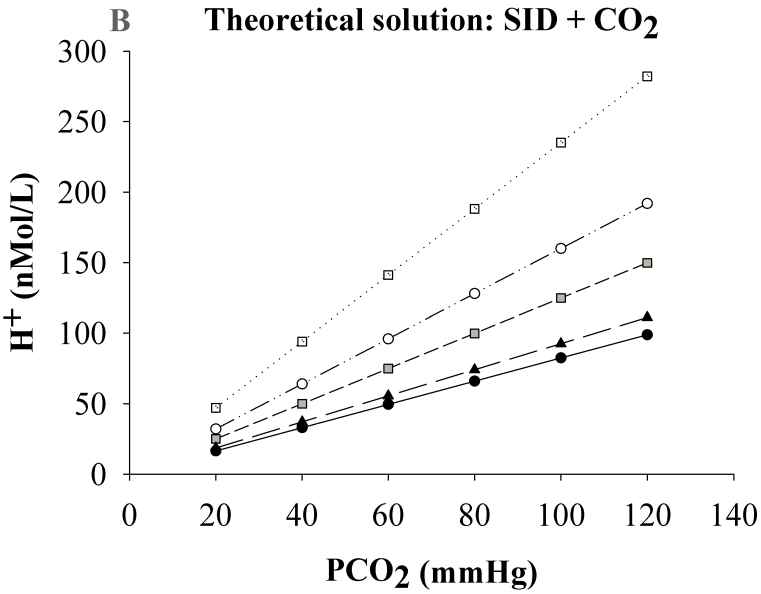
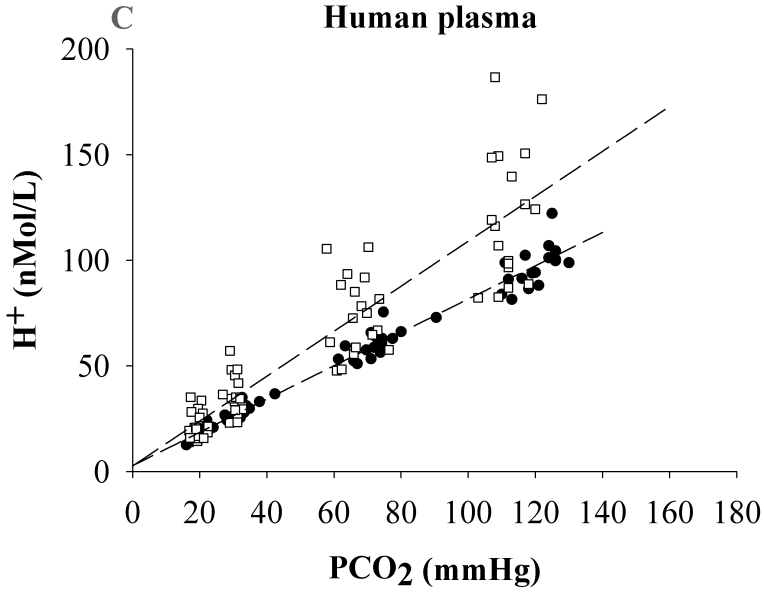


	H^+_{20} (nMol/L)	Alb (g/dL)	β_{PS} (mmHg/nMol/L)	$\beta_{PS(pH)}$ (mmHg)	β_{VS} (mEq/L)
⊞	47	9.3	1.10	217	24.9
○	32	6.6	1.40	198	26.3
▢	25	4.7	1.61	185	26.9
▲	18.5	2.8	1.63	157	18.0
●	16.5	2.1	1.68	150	13.7



	H^+_{20} (nMol/L)	SID (mEq/L)	β_{PS} (mmHg/nMol/L)	$\beta_{PS(pH)}$ (mmHg)	β_{VS} (mEq/L)
⊞	47	10.3	0.43	129	0
○	32	15.1	0.63	129	0
▢	25	19.3	0.80	129	0
▲	18.5	26.1	1.08	129	0
●	16.5	29.3	1.21	129	0



	□ Septic patients	● Healthy subjects
H^+_{20} (nMol/L)	20.7 [16.7-27.5]	17.0 [16.0-19.6]
Albumin (g/dL)	3.0 [2.8-3.2]	4.7 [4.6-4.9]
SID (mEq/L)	36.0 [32.1-40.0]	41.5 [40.1-43.1]
β_{PS} (mmHg/nMol/L)	0.96 [0.78-1.16]	1.28 [1.23-1.34]
β_{VS} (mEq/L)	2.41 [1.50-2.65]	3.96 [2.95-4.36]