CALCULATION OF ISOELECTRIC POINTS.

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The formula for calculation of the isoelectric point of a simple mono-basic, mono-acidic amphoteric substance is as follows:

$$I = \sqrt{\frac{K_a}{K_b} K_w} = \sqrt{K_a k_b} \quad \text{or } pI = \frac{pK_a + pk_b}{2} \qquad (1)$$

Where I = the isoelectric point (hydrogen ion concentration at that point).

K_a = the equilibrium constant of the acid.

K_b = the equilibrium constant of the base.

K_w = the equilibrium constant of the water at the temperature under consideration.

$$k_b = \frac{K_w}{K_b}$$

It will be shown in this paper that this formula has an even wider application since in more complex ampholytes (as proteins) the isoelectric point may be approximately calculated from the equilibrium constants of the *strongest acid group* and the *strongest basic group* by the above equation.

A more exact equation is:[1]

$$I = \sqrt{\frac{K_{a1} + K_{a2} + K_{a3} \; . \; . \; . \; . + K_{am}}{K_{b1} + K_{b2} + K_{b3} \; . \; . \; . \; . + K_{bn}} K_w} = \sqrt{\frac{\Sigma K_a}{\Sigma K_b} K_w} \qquad (14)$$

From this equation it is obvious that when the numerical value of the weaker K_as and K_bs is negligible in comparison with the strongest K_a and K_b, the equation resolves itself into equation (1). Thus if the second pK_a is *one pH unit* away from the first, it

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[1] Even this latter equation is approximate, but it is accurate enough for practical use since the error is very small. The correct expression as applied to a di-basic, di-acidic ampholyte is given later in the paper.

may be neglected with small error. The weaker basic K_bs may be similarly disregarded.

The reason for assuming that the weaker acid and basic groups can be neglected may be demonstrated in the case of aspartic acid. Its dissociation is represented in Fig. 1. As determined by the authors, aspartic acid at 30°C. has the following constants.[2]

$$\begin{aligned} pK_{a1} &= 3.63 & K_{a1} &= 2.35 \cdot 10^{-4} \\ pK_{a2} &= 9.47 & K_{a2} &= 3.39 \cdot 10^{-10} \\ pk_b &= 1.90 & k_b &= 1.26 \cdot 10^{-2} \\ pK_b\ (= pK_w - pk_b) &= 11.82 & K_b &= 1.50 \cdot 10^{-12} \\ pK_w \text{ at } 30°\text{C.} &= 13.725 & K_w &= 1.89 \cdot 10^{-14} \end{aligned}$$

These values were calculated from the titration data given in Table I, which were obtained for aspartic acid (molecular weight = 133) at 30°C., in 0.1 molar solution. The titration was at constant volume. A water-jacketed electrode was used which will be described in another publication. Near the isoelectric point, the values were obtained from solutions which were supersaturated.

If we consider only the stronger acid group, the calculation of the isoelectric point by equation (1) gives pI = 2.76. If this is the isoelectric point, the calculated hydrogen ion concentration for the dissolved substance in 0.1 N solution is pH 2.80.

In Fig. 1 the point I (indicated by the arrow) is the calculated isoelectric point where only the stronger acid group is considered. Calculation of the isoelectric point from equation (2) gives:

$$I = \sqrt{\frac{0.000235 + 0.000000000339}{1.50 \times 10^{-12}} \times 1.89 \times 10^{-14}}$$

$$pI = 2.76$$

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[2] It will be shown in a later publication that the solubility of the *undissociated molecule* of aspartic acid has a constant value of 0.034 mol per liter at 25°C.

The *total* solubility can be calculated from the ratio:

$$S = \frac{0.034}{(1 - \alpha_a)\ (1 - \alpha_b)} = \text{total solubility.}$$

Where α_a and α_b equal the degree of dissociation of the first acid group and the basic group, respectively.

The value $(1 - \alpha_a)\ (1 - \alpha_b)$ represents the fraction of molecules not ionized.

TABLE I.

$\frac{B}{C} = \frac{-A}{C}$ Molar equivalents of base.	$B' = -A' = \frac{B+H}{C}$ Corrected for acid required to bring water to the same pH.	pH	pk_b	pK_{a1}	pK_{a2}
−1.0	−0.69	1.51	1.87		
−0.9	−0.64	1.61	1.88		
−0.8	−0.60	1.70	1.90		
−0.7	−0.53	1.82	1.90		
−0.6	−0.49	1.95	1.96		
−0.5	−0.42	2.09	(2.00)		
−0.4	−0.34	2.18	1.95		
−0.3	−0.25	2.31	1.92		
−0.2	−0.16	2.43	1.87		
−0.1	−0.07	2.60	(2.01)		
0	0.02	2.72	1.87		
+0.3		3.31		3.60	
+0.4		3.50		3.63	
+0.5		3.67		3.64	
+0.6		3.84		3.65	
+0.7		4.02		3.64	
+0.8		4.27		3.66	
+0.9		4.57		3.62	
+0.95		4.87		3.59	
+1.0		6.93			
+1.05		8.16			9.44
+1.10		8.48			9.43
+1.2		8.86			9.46
+1.3		9.11			9.48
+1.4		(9.47)			(9.64)
+1.5		9.580			(9.58)
+1.6		9.65			9.48
+1.7		9.85			9.48
+1.8		10.08			9.48
+1.9		10.36			(9.41)
+2.0		10.75			
Average of best values.............			1.90	3.63	9.47

pk_b is calculated from the equation $pk_b = pH + \log \frac{HA' + K_{a1}(1 + A')}{H(1 - A') - K_{a1} A'}$

pK_{a1} " " " " " $pK_{a1} = pH + \log \frac{k_b(1 - B') - HB'}{k_b B' + H(1 + B')}$

pK_{a2} " " " " " $pK_{a2} = pH + \log \frac{2 - B'}{B' - 1}$

The derivation of these formulas will be given in a future publication.

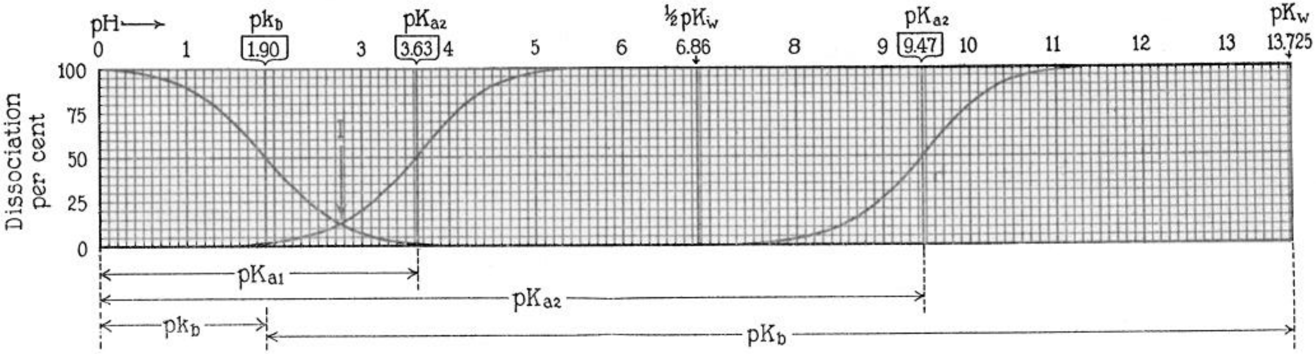

FIG. 1. Dissociation diagram of aspartic acid at 30°C.

This is the same value obtained by using equation (1) since the term K_{a2} is negligible in comparison with K_{a1}.

At the isoelectric point the first acid group is 15 per cent ionized, while the ionization of the second acid group is only 0.00002 per cent when calculated by the formula:

$$pH = pK + \log \frac{\alpha}{1 - \alpha}$$

In other words, the first acid group of aspartic acid is nearly *one million times* as much ionized as the second. Hence the second may be neglected in calculating the isoelectric point.

The following values illustrate the relative accuracies of equations (1), (14), and (15).

Equation (1). $$I = \sqrt{K_{a1} k_{b1}} = \sqrt{\frac{K_{a1}}{K_{b1}} K_w} \text{ or } pI = \frac{pK_{a1} + pk_{b1}}{2},$$

where only the strongest acid and basic groups are considered.

Equation (14). $$I = \sqrt{\frac{K_{a1} + K_{a2}}{k_{b1} + k_{b2}} k_{b1} k_{b2}} = \sqrt{\frac{K_{a1} + K_{a2}}{K_{b1} + K_{b2}} K_w}$$

Equation (15)
(exact formula).

$$I = \sqrt{\frac{K_{a1} + K_{a2}}{k_{b1} + k_{b2}} k_{b1} k_{b2} + K_{a1} K_{a2} \left(1 + \frac{2k_{b1} k_{b2}}{H(k_{b1} + k_{b2})}\right) - \frac{H_2}{k_{b1} + k_{b2}} (K_{a1} + K_{a2} + 2H)}$$

as applied to two hypothetical di-basic, di-acidic ampholytes having the pK and pk values indicated.

TABLE II.

Case No.	pK_{a1}	pK_{a2}	pk_{b1}	pk_{b2}	pI = isoelectric point.			
					Equation (1).	Equation (14).	Equation (15).	True value.
1	3	5	2	1.00	2.500	2.520	2.531	2.531
2	4	10	2	1.43	3.000	3.052	3.060	3.060

Conception of Isoelectric Point.

In this paper the *isoelectric point* of an amphoteric substance will be interpreted to signify *that hydrogen ion concentration at which it is ionized equally as an acid and as a base.*

The isoelectric point may also be considered as the point of *minimum dissociation.* It will be seen later that both definitions lead to the same formula.

It is at this point that the conductivity will be a minimum, if the mobilities of the ampholyte are the same in the anion and cation forms.

Other properties such as optimum precipitation and agglutination, minimum viscosity, swelling, and solubility are associated with this degree of acidity or alkalinity which we call the isoelectric point.

It must be kept in mind that this point is *not* necessarily the same as the hydrogen ion concentration of a pure solution of the ampholyte.

At the *isoelectric point* the condition is represented by the equation

$$\alpha_{b1}C + \alpha_{b2}C + \alpha_{b3}C \ldots + \alpha_{bn}C = \alpha_{a1}C + \alpha_{a2}C + \alpha_{a3}C \ldots + \alpha_{am}C$$

or

$$\Sigma\alpha_b = \Sigma\alpha_a$$

while the hydrogen ion concentration of a solution of ampholyte is represented by

$$[H^+] + \Sigma\alpha_bC = [OH^-] + \Sigma\alpha_aC$$

where

α_b represents the degree to which a basic group is ionized.
α_a represents the degree to which an acid group is ionized.
C represents the concentration of ampholyte.
$[H^+]$ and $[OH^-]$ have the usual significance.

It will be seen that the *isoelectric point* as defined is *constant* and *independent of the concentration,* while the *hydrogen ion concentration* of the pure ampholyte solution is obviously a *variable* since it is a function of the concentration.

Except in the case where the isoelectric point happens to be at the "neutral point" of water, it is *never* identical with the hydrogen ion concentration of the dissolved substance. However, the difference is quite insignificant in concentrated solutions and is of notable magnitude in only quite dilute solutions.

Mode of Ionization of Ampholytes.

The following derivations of formulas for the calculation of isoelectric points are based on the assumption that the ionization of each group (acid or basic) takes place *independently* of the *degree* of ionization of other groups in the molecule. It is understood that the numerical value of each K may be influenced by the relative position of other acidic groups. Mathematically, we may treat an ampholyte as if it were a mixture of a number of monovalent acids and bases having the same respective dissociation constants as the various groups of the ampholyte.

This conception will be more fully discussed in another publication. It is in accord with experimental data as far as the mass law is applicable.

Derivation of Formulas for the Calculation of the Isoelectric Point of Poly-Acidic, Poly-Basic Ampholytes.

If we define the isoelectric point of a substance as *that hydrogen ion concentration at which it is ionized equally as an acid and as a base,* we may represent this relation as follows:

$$\alpha_{b1}C + \alpha_{b2}C + \alpha_{b3}C \dots + \alpha_{bn}C = \alpha_{a1}C + \alpha_{a2}C + \alpha_{a3}C \dots + \alpha_{am}C \quad (2)$$

Where C represents the concentration of the ampholyte and the various values of α_b and α_a represent the extent to which the various basic and acid groups are ionzed.

This may be written

$$\Sigma_n \alpha_b C = \Sigma_m \alpha_a C \quad (3)$$

or

$$\Sigma \alpha_b = \Sigma \alpha_a \quad (4)$$

that is, *the sum of the ionized fractions of basic groups equals the sum of the ionized fractions of the acid groups of an ampholyte* at its isoelectric point. It will be noted that the concentration factor (C) cancels out, thus making the isoelectric point *independent of the concentration.*

That this point is *not* identical with the hydrogen ion concentration of a *solution* of the *pure ampholyte* will be seen from the fact that this latter condition is represented by

$$[H^+] + \Sigma\alpha_b C = [OH^-] + \Sigma\alpha_a C \quad (5)$$

(for the sum of the positive ions in a solution must equal the sum of the negative ions).

Except in the special case where the isoelectric point is at the "neutral" point of water $[H^+]$ does not equal $[OH^-]$ and the pH of a pure solution of ampholyte lies between its isoelectric point and the neutral point of water. The exact pH is a function of concentration and in concentrated solutions its deviation from the isoelectric point is within experimental error.

1. Isoelectric Point of a Simple Ampholyte.

For the calculation of the pH at which

$$\Sigma\alpha_b = \Sigma\alpha_a \tag{4}$$

it has been customary to use the equation

$$I = \sqrt{\frac{K_a}{K_b} K_w} \tag{1}$$

where $n = m = 1$; that is, where there is but one acid group and one basic group.

This equation will be later deduced from a more general equation applying to cases in which there are more acid and more basic groups.

2. Isoelectric Point of a Poly-Basic, Poly-Acid Ampholyte.

The law of mass action for any mono-basic acid may be expressed as follows:

$$K_a = [H^+] \frac{\alpha_a}{1 - \alpha_a} \tag{6}$$

where K_a = the equilibrium constant,
$[H^+]$ = the hydrogen ion concentration,
α_a = the fraction of the acid in the ionized state.

Hence:

$$\alpha_a = \frac{K_a}{H + K_a} \tag{7}$$

Similarly, for a base:

$$K_b = [OH^-] \frac{\alpha_b}{1 - \alpha_b} \tag{8}$$

and

$$\alpha_b = \frac{K_b}{[OH^-] + K_b} \tag{9}$$

It will be recalled that the pK of an acid is the hydrogen ion concentration (expressed in terms of pH) at which the acid is half ionized.

For, if

$$pK_a = pH = -\log K_a = -\log H$$

then

$$K_a = [H^+]$$

and

$$\frac{K_a}{[H^+]} = \frac{\alpha_a}{1 - \alpha_a} = 1$$

or

$$\alpha_a = 1 - \alpha_a \text{ and } \alpha_a = 0.5$$

So that at this point the numerical values of the dissociation constant and the hydrogen ion concentration are equal. Similarly, the pK of a base is equal to the p[OH⁻] or the negative logarithm of the *hydroxyl ion concentration* at which the base is half dissociated.

However, since we are accustomed to indicate acidity and alkalinity in terms of pH, pk_b may be used to indicate the pH at which the base is half dissociated.

The relation is

$$pk_b = pK_w - pK_b \tag{10}$$

or

$$k_b = \frac{K_w}{K_b} \text{ and } K_b = \frac{K_w}{k_b}$$

Substituting this in equation (9) and placing $[OH^-] = \frac{K_w}{[H^+]}$ we get

$$\alpha_b = \frac{[H^+]}{[H^+] + k_b} \tag{11}$$

Let us now take the case of an ampholyte with two acid and two basic groups whose ions are represented by α_{a1}, α_{a2}, α_{b1}, and α_{b2}. Their dissociation constants are: K_{a1}, K_{a2}, K_{b1}, and K_{b2} or K_{a1}, K_{a2}, $\frac{K_w}{k_{b1}}$, and $\frac{K_w}{k_{b2}}$. From equation (4) $\alpha_{b1} + \alpha_{b2} = \alpha_{a1} + \alpha_{a2}$; then from equations (7) and (11)

$$\frac{[H^+]}{[H^+] + k_{b1}} + \frac{[H^+]}{[H^+] + k_{b2}} = \frac{K_{a1}}{[H^+] + K_{a1}} + \frac{K_{a2}}{[H^+] + K_{a2}} \tag{12}$$

Solving this equation for [H+] involves a fourth power equation, so let us solve it in an approximate form and later derive the exact expression.

By way of approximation this may be written

$$\frac{[H^+]}{k_{b1}} + \frac{[H^+]}{k_{b2}} = \frac{K_{a1}}{[H^+]} + \frac{K_{a2}}{[H^+]} \qquad (13)$$

Solving

$$I = [H^+] = \sqrt{\frac{K_{a1} + K_{a2}}{K_{b1} + K_{b2}}\, k_{b1} \cdot k_{b2}} \qquad (14a)$$

or

$$I = [H^+] = \sqrt{\frac{K_{a1} + K_{a2}}{K_{b1} + K_{b2}}\, K_w} \qquad (14b)$$

This is an equation which gives the approximate value of the isoelectric point of a di-basic, di-acidic ampholyte (see Table I). The exact solution of equation (12) gives (in terms of k_{b1} and k_{b2}):

$$I = \sqrt{\frac{K_{a1} + K_{a2}}{k_{b1} + k_{b2}}\, k_{b1}\ k_{b2} + K_{a1} \cdot K_{a2} \left(1 + \frac{2k_{b1} \cdot k_{b2}}{H(k_{b1} + k_{b2})}\right) - \frac{H^2}{k_{b1} + k_{b2}} (K_{a1} + K_{a2} + 2H)} \qquad (1$$

or (in terms of K_{b1} and K_{b2}):

$$I = \sqrt{\frac{K_{a1} + K_{a2}}{K_{b1} + K_{b2}}\, K_w + K_{a1}\ K_{a2} \left(1 + \frac{2K_w}{H(K_{b1} + K_{b2})}\right) - \frac{H^2 K_{b1} K_{b2}}{K_w (K_{b1} + K_{b2})} (K_{a1} + K_{a2} + 2H)} \qquad (1$$

It will be seen that the first terms in these equations are identical with the approximate equations (14a) and (14b). Hence it is to be expected that the sum of the remaining terms should be small or negligible. This is the case, and it will be seen from Table I that the error resulting from use of the simpler form is very small.

If it is desired to obtain a value for the isoelectric point which is of such accuracy that the error is entirely negligible, it is only necessary to obtain the approximate value of [H+] according to equation (14a) or (14b) and substitute this value for [H+] in equation (15a) or (15b). The value thus obtained is sufficiently accurate to be well within experimental error.

For an ampholyte having *m* acid groups and *n* basic groups, we saw that (equation (2)):

$$\alpha_{b1}C + \alpha_{b2}C \ldots . + \alpha_{bn}C = \alpha_{a1}C + \alpha_{a2}C \ldots . + \alpha_{am}C$$

or

$$\alpha_{b1} + \alpha_{b2} \ldots . + \alpha_{bn} = \alpha_{a1} + \alpha_{a2} \ldots . + \alpha_{am}C$$

Thus from equations (7) and (11)

$$\frac{[H^+]}{[H^+] + k_{b1}} + \frac{[H^+]}{[H^+] + k_{b2}} \ldots . + \frac{[H^+]}{[H^+] + k_{bn}} = \frac{K_{a1}}{[H^+] + K_{a1}} + \frac{K_{a2}}{[H^+] + K_{a2}} \ldots . + \frac{K_{am}}{[H^+] + K_{am}}$$

making the same approximation as in equation (13)

$$\frac{[H^+]}{k_{b1}} + \frac{[H^+]}{k_{b2}} \ldots . + \frac{[H^+]}{k_{bn}} = \frac{K_{a1}}{[H^+]} + \frac{K_{a2}}{[H^+]} \ldots . + \frac{K_{am}}{[H^+]}$$

Hence:

$$I = [H^+] = \sqrt{\frac{K_{a1} + K_{a2} \ldots . + K_{am}}{\frac{1}{k_{b1}} + \frac{1}{k_{b2}} \ldots . + \frac{1}{k_{bn}}}} = \sqrt{\frac{\Sigma K_a}{\Sigma \frac{1}{k_b}}} \qquad (14)$$

or

$$I = [H^+] = \sqrt{\frac{K_{a1} + K_{a2} \ldots . + K_{am}}{K_{b1} + K_{b2} \ldots . + K_{bn}} K_w} = \sqrt{\frac{\Sigma K_a}{\Sigma K_b} K_w}$$

This is the general approximate formula for the isoelectric point of an ampholyte with any number (*m*) of acid groups and any number (*n*) of basic groups (see Table I).

Equation (14) may be derived in a different manner on the assumption that at the isoelectric point the sum of all the ions ($\Sigma\alpha C$) is at a minimum. As in equation (13) we will take the approximate concentration of an acid ion, $\frac{K_aC}{[H^+]}$, and of a basic ion, $\frac{[H^+]K_bC}{K_w}$.

Then at the point of minimum concentration of ions:

$$\frac{d\Sigma\alpha C}{dH} = \frac{d}{dH}\left(\frac{K_{a1}C}{H} + \frac{K_{a2}C}{H} \ldots . + \frac{K_{am}C}{H} + \frac{K_{b1}CH}{K_w} + \frac{K_{b2}CH}{K_w} \ldots . + \frac{K_{bn}CH}{K_w}\right) = 0$$

Then

$$\frac{C}{H^2}(K_{a1}+K_{a2}\ldots.+K_{am})+\frac{C}{K_w}(K_{b1}+K_{b2}\ldots.+K_{bn})=0$$

or

$$\frac{\Sigma K_b}{K_w}-\frac{\Sigma K_a}{H^2}=0$$

Hence

$$I=H=\sqrt{\frac{K_{a1}+K_{a2}\ldots.+K_{am}}{K_{b1}+K_{b2}\ldots.+K_{bn}}K_w}=\sqrt{\frac{\Sigma K_a}{\Sigma K_b}K_w} \qquad (14)$$

which is the same formula as the one obtained algebraically.

Substitution of this value in the second derivative

$$\frac{d^2\Sigma\alpha C}{dH^2}=\frac{2\Sigma K_a C}{H^3}$$

gives a positive value.

Hence equation (14) represents a minimum.

Position of Acid and Basic pK's.

It is not to be expected that all the acid pK's should occur above the isoelectric point and all the basic pk's (= $pK_w - pK_b$) should fall below this point. The above formulas apply to all cases, whether all the pK_a's are above and the pk_b's are below the isoelectric point, or whether there are some of each on both sides.

In any case where an acid pK does fall below the isoelectric point, there must be at least one basic pk above it.

It was found on titration of both aspartic acid (see Fig. 1) and glutamic acid that the solubility was lowest at the isoelectric point and increased on changing the pH in either direction. This may be ascribed to the insolubility of the undissociated molecule which is present in the largest proportion at the isoelectric point.[2]

Isoelectric Range.

While in aspartic acid, glutamic acid, and many proteins, the isoelectric point marks a sharp boundary where there is a definite change in behavior, this is not the case with all ampholytes. In the above cases the sharp change results from the proximity of the acid and basic pK's to the isoelectric point. If, on the other hand, we consider glycine, alanine, leucine, etc., it will be seen that there is considerable range over which the ampholyte is dissociated. Thus between pH 4.5 and 8, the above three amino-acids are undissociated, exert no buffer effect, and behave in solution much as non-electrolytes. If we calculate the isoelectric point for such a substance, the value has no practical significance. There is in reality an isoelectric *range* from pH 4.5 to 8, in which there is no change in properties either physically or from the standpoint of ionization.

It is more proper, therefore, to speak of an isoelectric point only when pK_{a1} is less than 4 pH units above pk_{b1}.

SUMMARY.

1. The usual formula for single ampholytes:

$$I = \sqrt{\frac{K_a}{K_b} K_w} = \sqrt{K_a \cdot k_b}$$

may be used to calculate the approximate isoelectric point of poly-acidic, poly-basic ampholytes without much error by using the K_a and K_b of the strongest acid and strongest basic group.

2. A more exact expression is:

$$I = \sqrt{\frac{K_{a1} + K_{a2} \ldots . + K_{am}}{K_{b1} + K_{b2} \ldots . + K_{bn}} K_w} = \sqrt{\frac{K_{a1} + K_{a2} \ldots . + K_{am}}{\frac{1}{k_{b1}} + \frac{1}{k_{b2}} \ldots . + \frac{1}{k_{bn}}}}$$

$$I = \sqrt{\frac{\Sigma K_a}{\Sigma K_b} K_w} = \sqrt{\frac{\Sigma K_a}{\Sigma \frac{1}{k_b}}}$$

This will give a value with very small error.

3. The exact expression for the value of I is given.

4. The ionization of each acid or basic group is assumed to be independent of the degree to which all other groups are ionized at a given pH.

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