

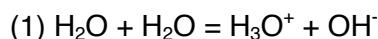
A General Tutorial on Acid-Base Chemistry as a Basis for Understanding the Diamond Analytics Flare Mixed-Mode Column

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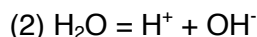
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In this tutorial we review the basic concepts of the auto-ionization of water, K_w , pH and pOH, the dissociation of acids and bases in water, K_a and K_b , pK_a and pK_b , and the Henderson-Hasselbach equation. A more complete explanation of this information can be found in most general chemistry books. The purpose of this tutorial is to provide a general, conceptual basis to the chromatographer interested in using the Diamond Analytics Flare mixed mode column. A more specific tutorial on the chemistry of the Flare column and possible analytes that might be separated on it is given in an application note entitled: 'Guidelines for Understanding the Retention Mechanism of the Diamond Analytics Flare Mixed-Mode Column'.

A. The auto-ionization of water. In pure water, some of the water molecules will occasionally collide in such a way that a proton is exchanged between them:



Note that this proton may also be referred to as a hydrogen ion, a hydronium ion, H^+ , or H_3O^+ . The process in Equation 1 is referred to as the auto-ionization (self-ionization) of water. It can also be represented in the following equivalent, but abbreviated, way:



B. The K_w . Because of its auto-ionization, water will always have present at least small amounts of the hydrogen ion (H^+) and hydroxide ion (OH^-) and these concentrations: $[\text{H}^+]$ and $[\text{OH}^-]$ (concentrations in chemistry are represented by placing a chemical species in square brackets) will be equal in pure water. At room temperature, $[\text{H}^+] = [\text{OH}^-] = 1.0 \times 10^{-7} \text{ M}$. Most solutions (not pure water) will have hydrogen ion ($[\text{H}^+]$) and hydroxide ion ($[\text{OH}^-]$) concentrations that are not equal. However, $[\text{H}^+]$ and $[\text{OH}^-]$ cannot be varied independently of each other, but rather are constrained by the following relationship:



Where 1.0×10^{-14} is the equilibrium constant for the auto-ionization of water (K_w) at room temperature, and the concentration of water is always omitted from these types of equilibrium expressions. Equation 3 follows directly from Equation 2. An equivalent form of Equation 3, which would follow from Equation 1, can also be written:

$$(4) K_w = [\text{H}_3\text{O}^+][\text{OH}^-] = 1.0 \times 10^{-14}$$

Thus, we see that if either $[\text{H}^+]$ or $[\text{OH}^-]$ goes down, the other must go up (they are inversely related), e.g., if $[\text{H}^+] = 1.0 \times 10^{-9} \text{ M}$ then $[\text{OH}^-] = 1.0 \times 10^{-5} \text{ M}$.

C. **The pH and pOH.** The pH and pOH of an aqueous solution are defined as the negative log of the hydrogen ion and hydroxide ion concentrations, respectively:

$$(5) \text{pH} = -\log[\text{H}^+] = -\log[\text{H}_3\text{O}^+]$$

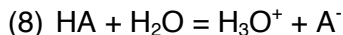
$$(6) \text{pOH} = -\log [\text{OH}^-]$$

It follows that if we take the negative log of Equation 3 or Equation 4 and do a little algebra we obtain:

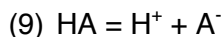
$$(7) \text{pH} + \text{pOH} = 14.00$$

So for a hydrogen ion concentration of $1.0 \times 10^{-7} \text{ M}$, $\text{pH} = -\log[1.0 \times 10^{-7}] = 7.00$. A solution with a pH value of 7.00 is said to be neutral. If the hydrogen ion concentration is greater than $1.0 \times 10^{-7} \text{ M}$, the solution is acidic and its pH is less than 7.00, e.g., if $[\text{H}^+] = 1.0 \times 10^{-6} \text{ M}$, then $\text{pH} = 6.00$. On the other hand, if $[\text{H}^+]$ is less than $1.0 \times 10^{-7} \text{ M}$, the solution is basic and its pH is greater than 7.00, e.g., if $[\text{H}^+] = 1.0 \times 10^{-12} \text{ M}$, then $\text{pH} = 12.00$. From Equation 7, we see that if the pH of a solution is equal to 4.00, then the pOH for that solution is 10.00.

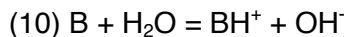
D. **Dissociation of Acids and Bases in Water.** Strong acids and bases dissociate completely in water – they are strong electrolytes, and weak acids and bases, which we will represent as HA and B, generally only dissociate to a very small extent in water – they are weak electrolytes. The dissociations of a weak acid, HA, (Equations 8 and 9) and of a weak base, B, (Equation 10) in water are given as follows:



or

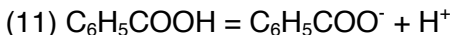


and



Equation 8 is more complete than Equation 9 in the sense that it shows water acting as a base to accept a proton from the weak acid, HA. However, this process is often represented in the abbreviated form given in Equation 9. Equation 10 shows a weak

base, B, removing a proton from water to form the protonated form of B, BH⁺, and also OH⁻. In Equations 8 and 9, A⁻ is referred to as the conjugate base of the weak acid HA, and in Equation 10, BH⁺ is referred to as the conjugate acid of the weak base B. For an actual weak acid, benzoic acid (C₆H₅COOH), the dissociation represented in Equation 9 is as follows:



Where, C₆H₅COOH and C₆H₅COO⁻ represent the weak acid, HA, and its conjugate base, A⁻, respectively. For an actual weak base, ammonia (NH₃), the dissociation represented in Equation 10 is as follows:



Obviously, NH₄⁺/NH₃ are the conjugate acid/base pair here.

E. **The K_a and K_b.** K_a and K_b are the equilibrium constants for the dissociation of a weak acid and a weak base, respectively. Note that subscripts like 'w', 'a', or 'b' are often added to an equilibrium constant to designate the type of equilibrium under consideration. For Equations 8, 9, and 11, mathematical expressions for K_a can be written as follows:

$$(13) K_a = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}]}$$

$$(14) K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

and

$$(15) K_a = \frac{[\text{C}_6\text{H}_5\text{COO}^-][\text{H}^+]}{[\text{C}_6\text{H}_5\text{COOH}]}$$

Note: Each acid will have its own unique value of K_a, and that the larger the K_a, the greater the dissociation of HA, i.e., [H⁺] and [A⁻] will be larger for a larger value of K_a so the solution will be more acidic. The K_a values for many common weak acids are in the range of about 10⁻⁴ to 10⁻¹⁰. For example, the K_a values for formic acid (HCOOH), acetic acid (CH₃COOH) and phenol (C₆H₅OH) are 1.8 x 10⁻⁴, 1.8 x 10⁻⁵ and 1.6 x 10⁻¹⁰, respectively. K_a values do not have units. Obviously formic acid is the strongest of these three weak acids and phenol is the weakest.



For Equations 10, and 12, mathematical expression for K_b can be written as follows:

$$(16) K_b = \frac{[BH^+][OH^-]}{[B]}$$

$$(17) K_b = \frac{[NH_4^+][OH^-]}{[NH_3]}$$

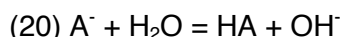
F. **The pK_a and pK_b .** As we have seen, K_a and K_b values can vary over many orders of magnitude. Accordingly, pK_a and pK_b are often used in place of K_a and K_b values, where the pK_a and pK_b are defined as:

$$(18) pK_a = -\log K_a$$

$$(19) pK_b = -\log K_b$$

For example, acetic acid has a K_a value of 1.8×10^{-5} , which gives it a pK_a value of $-\log(1.8 \times 10^{-5})$, which is 4.75. Similarly, the K_a value for phenol is 1.6×10^{-10} so its K_a value is 9.80. Thus we see that the lower the pK_a value of an acid (or a base) the stronger it is.

There is a useful relationship between the K_a and K_b values for a conjugate acid/base pair, which we represent as HA and A^- . If we rewrite Equations 10 and 16 in terms of A^- , the conjugate base of HA, we get:



and

$$(21) K_b = \frac{[HA][OH^-]}{[A^-]}$$

Now if we multiply the expression for K_a for HA (Equation 14) by the expression for K_b for A^- (Equation 21) we obtain:

$$(22) K_a * K_b = \frac{[H^+][A^-]}{[HA]} * \frac{[HA][OH^-]}{[A^-]} = [H^+][OH^-] = K_w = 1.0 \times 10^{-14}$$

That is:



$$(23) \quad K_a \times K_b = K_w = 1.0 \times 10^{-14}$$

For example, because ammonia (NH₃) has a K_b of 1.8×10^{-5} , the K_a for its conjugate acid (NH₄⁺) is 5.6×10^{-10} (as derived from Equation 23).

If we take the negative log of both sides of Equation 23 and do a little algebra we obtain another useful relationship:

$$(24) \quad pK_a + pK_b = pK_w = 14.00$$

G. The Henderson-Hasselbach Equation. The Henderson-Hasselbach equation is used to describe buffers, which are solutions that contain both a weak acid and its conjugate base. The Henderson-Hasselbach equation will help us better understand how pH influences the ionization state of analytes and the Flare mixed-mode column itself. This equation is derived in a few short steps from Equation 14 – we begin by taking the negative log of both sides of that equation:

$$(25) \quad -\log K_a = -\log \left(\frac{[H^+][A^-]}{[HA]} \right)$$

Using Equation 18 and expanding the right side of Equation 25 we obtain the following:

$$(26) \quad pK_a = -\log[H^+] - \log \left(\frac{[A^-]}{[HA]} \right)$$

We now use Equation 5 and do a little algebra to obtain the Henderson-Hasselbach equation:

$$(27) \quad pH = pK_a + \log \left(\frac{[A^-]}{[HA]} \right)$$

The Henderson-Hasselbach equation teaches us some important things about solutions that contain ionizable analytes. First, it tells us that when the concentration of the weak acid ([HA]) and its conjugate base ([A⁻]) are the same ([A⁻]/[HA] = 1), the pH of the solution is equal to the pK_a of the weak acid. In other words, the concentrations of HA and A⁻ are equal when half of the weak acid, HA, is deprotonated, or if we are starting with the weak base, A⁻, when half of it is protonated. Perhaps for a chromatographer, a better way to look at the Henderson-Hasselbach equation is to recognize that by changing the pH of a solution (mobile phase) one can change the ratio of [A⁻] and [HA]. Three cases are particularly important here:

- (i) if the pH of a solution equals the pK_a value for the weak acid then [A⁻] = [HA]

- (ii) if the pH of a solution is one pH unit above the pK_a for the weak acid ($pH = pK_a + 1$), then there will be ten times as much A^- in solution as HA, i.e., $[A^-] = 10*[HA]$
- (iii) if the pH of a solution is one pH unit below the pK_a for the weak acid ($pH = pK_a - 1$), then there will be ten times as much HA in solution as A^- , i.e., $[HA] = 10*[A^-]$.

Because in many cases chromatographic retention is strongly influenced by the protonation state of an analyte, the pH of the mobile phase can have a significant effect on retention and also selectivity in a separation. We see from the Henderson-Hasselbach equation that the protonation state of an analyte is particularly sensitive to the pH of the mobile phase when it is within plus or minus one pH unit of the pK_a for the analyte, i.e., $pH = pK_a \pm 1$. When the pH of the solution is either above or below this range, the analyte will be either mostly deprotonated or protonated, respectively.

As an example, the pK_a for formic acid is 3.74, which means that in an aqueous solution when:

- (i) the pH is 3.74 the concentrations of formic acid ($HCOOH$) and formate ($HCOO^-$) are equal
- (ii) the pH is 4.74, $[HCOO^-] = 10*[HCOOH]$
- (iii) the pH is 2.74, $[HCOOH] = 10*[HCOO^-]$
- (iv) the pH is greater than 4.74 the formic acid is mostly deprotonated
- (v) the pH is less than 2.74 the formic acid is mostly protonated

