

Lecture 4: Buffers & Polyprotic Acids

Reading in Campbell: Chapter 2.3-2.6

Key Terms:

- **pH = pKa + log[A⁻]/[HA]**
- **Titration: pKa determination**
- **Equivalence point, Inflection point**
- **Calculation of degree of protonation**
- **Buffers, buffer construction**
- **Polyprotic Acids**

4.1 Solving pH Problems:

The H&H equation has predictive value. Once you know the pK_a of an acid and the pH, you can predict [A⁻]/[HA]. One particular case is routinely used in biochemistry: Given a pH and pK_a of an acid, calculate the fraction of the acid that is protonated: f_{HA} = ([HA]/A_T) and the fraction that is deprotonated:

f_{A⁻} = ([A⁻]/A_T), where A_T is the total concentration of acid: A_T = [HA] + [A⁻].

Defining R = [A⁻]/[HA]

$$pH = pKa + \log([A^-]/[HA])$$

$$pH = pKa + \log(R)$$

$$pH - pKa = \log(R)$$

$$10^{(pH-pKa)} = R$$

Once R is found, the fraction protonated and deprotonated is obtained as follows:

$$[A^-]/[HA] = R$$

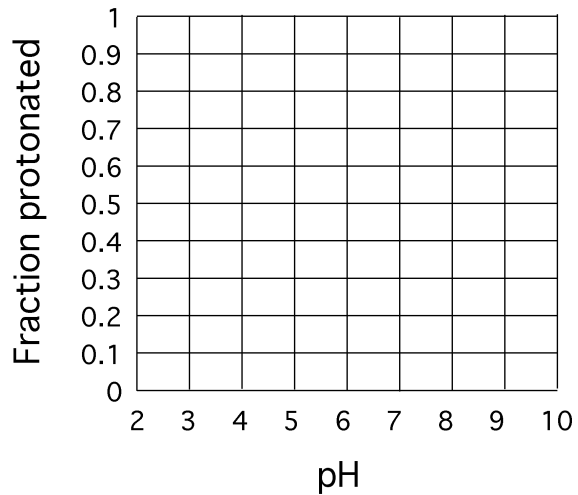
$$[A^-] = [HA]R$$

$$[A_T] = [A^-] + [HA]$$

$$[A_T] = [HA](1+R)$$

$$f_{HA} = \frac{[HA]}{[A_T]} = \frac{1}{(1+R)}$$

$$f_{A^-} = \frac{[A^-]}{[A_T]} = \frac{R}{(1+R)}$$



Using the ionization of the side chain of the amino acid Histidine as an example (pK_a = 6.0)

pH	R	F _{HA}
4	R = 10 ⁽⁴⁻⁶⁾ = 10 ⁻²	F _{HA} = 1/(1+0.01) = 0.99
5	R = 10 ⁽⁵⁻⁶⁾ = 10 ⁻¹	F _{HA} = 1/(1+0.1) = 0.91
6	R = 10 ⁽⁶⁻⁶⁾ = 10 ⁰	F _{HA} = 1/(1+1) = 0.5
7	R = 10 ⁽⁷⁻⁶⁾ = 10 ¹	F _{HA} = 1/(1+10) = 0.091
8	R = 10 ⁽⁸⁻⁶⁾ = 10 ²	F _{HA} = 1/(1+100) = 0.01

There are four general statements that are useful to remember:

1. When the $\text{pH} = \text{pK}_a$, $[\text{HA}] = [\text{A}^-]$.
2. When the pH is lower than the pK_a , $[\text{HA}] > [\text{A}^-]$.
3. When the pH is higher than the pK_a , $[\text{HA}] < [\text{A}^-]$.
4. A pH change of 1 leads to a 10 fold change in the ratio of $[\text{A}^-]/[\text{HA}]$.

4.2 Titration Curves

K_a values are usually measured by direct experiment, usually with a pH titration. Known amounts of a strong base (NaOH) are added to a solution of a weak acid and the pH is measured as the amount of NaOH is added. As the base is added it removes the proton from the acid, as well as increasing the pH .

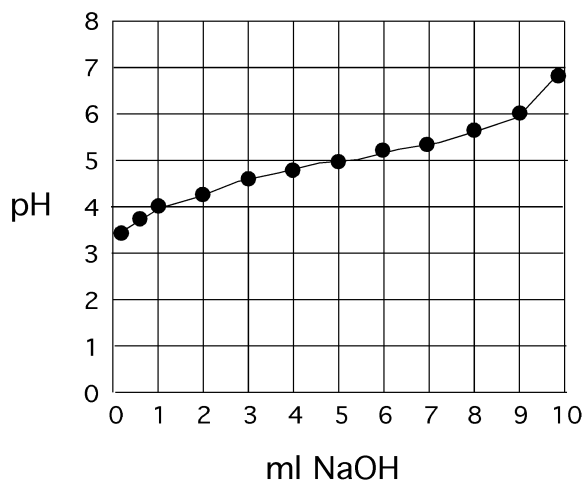
Inflection point ($\text{pH} = \text{pK}_a$): You can prove from the Henderson-Hasselbalch equation that the smallest change in pH due to addition of base occurs when the $\text{pH} = \text{pK}_a$; at this inflection point, the pH of the solution is the pK_a of the acid.

Equivalents: moles of base/moles of acid, the x-axis for titrations. Varies from 0 to 1 for monoprotic acids.

Equivalence point: Complete deprotonation of the weak acid occurs when the molar amount of base is equal to, or equivalent to, the molar amount of weak acid. This point in the titration is referred to as the equivalence point.

Example titration curve:

<u>ml NaOH</u>	<u>measured pH</u>
0.25	3.20
0.5	3.80
1	4.07
2	4.44
3	4.62
4	4.87
5	4.96
6	5.17
7	5.33
8	5.64
9	5.96
10	7.00



(Facts: Concentration of $\text{NaOH} = 1 \text{ M}$
Volume of solution that is titrated = 100 ml)

Possible acids (from Campbell table 2.6)

Pyruvate	$\text{pK}_a = 2.5$
Acetic Acid	$\text{pK}_a = 4.8$
Tris	$\text{pK}_a = 8.3$

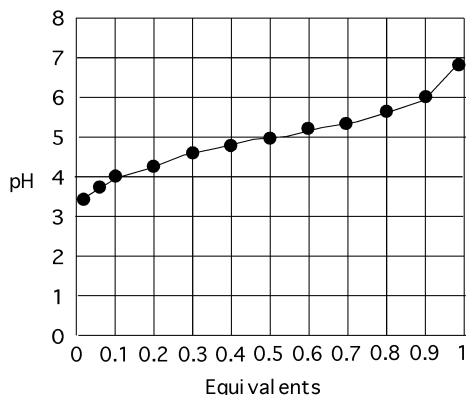
Questions:

1. What is the pK_a of the acid?
2. Which acid is it?
3. Give the x-axis scale in equivalents.

4.1 Buffers

A pH buffer is an acid that resists changes in the solution pH by absorbing or releasing protons. Buffers play an important role in cellular processes because they maintain the pH at an optimal level for biological processes. They are also widely used to control pH in laboratory processes.

All acids function well as buffers for pH values *within one pH unit* of their pKa value. Because the pKa of weak acids are near the physiological pH range, weak acids make good buffers.



Consider 1 L of a 10 mM solution of acetic acid at pH 4.7. At this pH, ~ 50% of the acid is ionized. If 1 mmole of a strong acid (e.g. HCl) is added, most of the protons that dissociate from the added acid would simply protonate the acetate ions, reducing their concentration from 5 mM to 4 mM. The resultant pH of the solution would be:

$$pH = pK_a + \log \frac{.004}{.006} = 4.52$$

The buffering capacity of a weak acid decreases as the dissociation becomes more complete (if base is added) or as it becomes more protonated (if acid is added). For example, acetic acid is a poor buffer at pH 3 or pH 7. The buffering capacity (ability to absorb protons) depends on both the concentration of the buffer and the difference between the pH and the pKa.

4.2 Buffer construction

Steps in making a buffer of concentration $[A^-]$:

1. Select a weak acid whose pKa is within one pH unit of the desired pH.
2. Calculate R, based on the difference between the desired pH and the pKa.
3. Calculate the required concentration of $[HA]$ and $[A^-]$ based on the total concentration of the buffer ($[HA]$).
4. To obtain this ratio of $[HA]$ to $[A^-]$:
 - a) Mix the indicated concentrations of the acid and its conjugate base to give the desired pH.
 - b) Alternatively, use $[A^-]$ amount of the *acid form* of the buffer and add sufficient strong base (e.g. NaOH) to obtain the desired concentration of $[A^-]$ by converting $[HA]$ to $[A^-]$ (i.e. the amount of base added equals $[A^-]$).
 - c) Alternatively, use $[A^-]$ amount of the *conjugate base form* of the buffer and add sufficient strong acid (e.g. HCl) to make the desired concentration of $[HA]$ (i.e. the amount of acid added equals $[HA]$).

Or, instead of performing any calculations whatsoever, use *either* the conjugate base or the acid form at a concentration of $[A^-]$, and slowly add the strong acid or base, respectively, until the measured pH is equal to the desired pH. This method of obtaining the desired buffer solution is most frequently used in practice!

Worked example:

Make 1L of a 0.1 M buffer solution at pH 5.0 using one of the following two buffers:

Buffer	pKa	MW (g/m)
Histidine	6.0 (side chain)	200 g/m (155 actual)
Pyruvic Acid	2.50	100 g/m (88 actual)

A. Which buffer would you use and why?

B. Calculate R at desired pH.

$$R = \frac{[A^-]}{[HA]} = 10^{(pH - pKa)}$$

$$f_{HA} = \frac{1}{(1 + R)} = \frac{[HA]}{[A_T]}$$

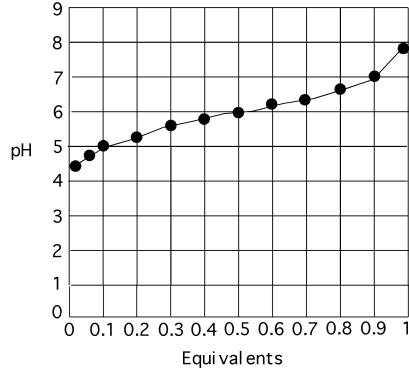
$$[HA] = f_{HA} [A_T]$$

$$f_{A^-} = \frac{R}{(1 + R)} = \frac{[A^-]}{[A_T]}$$

$$[A^-] = f_{A^-} [A_T]$$

C. Calculate [HA] and [A⁻] in moles.

D. Calculate [HA] and [A⁻] in grams and mix.



4.3 Polyprotic Acids

- Many compounds can release more than one proton in the pH range of 0-12. Examples are phosphate (pKa = 2.14, 7.20, 12.40), carbonate, dicarboxylic acids, as well as amino acids.
- If the pKas are separated by 2 or more pH units (i.e. phosphoric acid) each ionization can be treated separately.
- Depending on the structure of the polyprotic acid the pKa for each ionization can vary widely.

Consider the following examples:

Acid	pKa1	pKa2	pKa3
Phosphoric	2.14	7.20	12.40
Oxalic	1.23	4.19	-

Why do the pKa values change after the 1st proton is released?
In what direction do they change (weaker or stronger acid)?

