Lecture 4: Environmental pKa shifts, Charges, Titration curves & Buffers.

Goals:
- Predict environmental effect on pKa.
- Calculate charges on molecules with ionizable groups.
- Obtain pKa from a titration curve.
- Obtain concentration of weak acid from titrations.
- Understand the molecular nature of buffers.
- Buffer construction, monoprotic.

Chemical bonding and effects on pKa:

<table>
<thead>
<tr>
<th>Amine</th>
<th>pKa ~ 10 (Lys sidechain)</th>
<th>Easier to break an N-H bond versus an O-H bond, therefore a protonated amine is a stronger acid than an alcohol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>pKa ~ 14 (Ser, Thr sidechain)</td>
<td>Alcohol is a weak acid because of highly localized negative charge on the oxygen, deprotonated species is high energy.</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>pKa ~ 4.0 (Glu, Asp sidechain)</td>
<td>Negative charge delocalized over C=O, lower in energy, therefore a carboxylate is a stronger acid than an alcohol.</td>
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<tr>
<td>Carboxy</td>
<td>pKa ~ 2.0 group on an amino acid.</td>
<td>Electronegative nitrogen can withdraw some charge from the negatively charged carboxylate, giving a stronger acid than just the COOH.</td>
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Boltzmann Distribution: The relative populations \( n_b / n_a \) of two states depends on the energy difference between them, \( \Delta E = n_b / n_a \).

Chemical effects on Acid Strength:
The ionization of malonic acid is shown on the right. This diprotic acid has two different pKa values. Why?

Environmental effects on acid strength:
The environment of an ionizable group can change the pKa of that group, by affecting the energy of either the protonated or deprotonated states—remember, it is the relative energy difference between the HA and A- states that matters.
- If the HA state is stabilized relative to A-, the acid is weaker, it prefers to stay protonated (left panel). 
- If the HA state is destabilized, the acid will be stronger, since it prefers to be deprotonated (right panel).
- If the A- state is stabilized, the acid will be stronger since it prefers to deprotonate.
- If the A- state is destabilized, the acid will be weaker since it prefers to remain protonated.

Example: How will a positively charged environment affect the pKa of histidine (normally its pKa is 6.0)?

\[
K_a = \frac{[A^-][H^+]}{[HA]} \\
HA \rightleftharpoons A^- + H^+ \\
\]

Stronger Acid
Since \( E \) is lower than \( H^+ \),
Charge Calculations:

The overall charge on a molecule as a function of pH can be calculated by summing the contribution from each ionizable group:

i) Identify all ionizable groups on the molecule & their charge when protonated and deprotonated.

ii) Use the known pK_a of each group to determine the fraction protonated (f_{HA}) and deprotonated (f_A) at the required pH.

iii) Calculate the overall charge by summing the contribution of each group.

Example: What is the net charge on glycine at pH=8?

\[
\text{Zwitterion: a compound that is ionized, but has no net charge.}
\]

\[
\text{Isoelectric pH = pI, the pH where the net charge is zero.}
\]

Measuring the pK_a: Titration Curves

K_a values, or acidity constants, must be measured by direct experiment, usually with a pH titration. Known amounts of a strong base (NaOH) are added to a solution of 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 by removing free protons from water.

Key features of titration curves:

pK_a Determination (Inflection point): There is an inflection point when the weak acid is ½ deprotonated. Since the two forms of the acid (HA, A) are equal, the pH=pK_a of the acid.

Equivalence Point (Eq Pt): Complete deprotonation of the weak acid occurs when the amount of added base is equal to, or equivalent, to the total number of ionizable protons that were originally on the weak acid. This point in the titration is referred to as the equivalence point. The equivalence point can be used to determine the concentration of the acid.

Example: The titration curve on the right is the data obtained from a 100 mL solution of a weak acid. What is the concentration of the acid, assuming the NaOH concentration was 1 M.

\[
\text{mole NaOH = } \left( \frac{1 \text{ mole}}{0.01 \text{ L}} \right) = 0.01 \text{ mole}
\]

\[
\text{[Weak Acid]} = \frac{0.01 \text{ mole}}{0.1 \text{ L}} = 0.1 \text{ M}
\]
**Equivalents:** the x-axis can be converted to a scale of equivalents, defined as the ratio of the moles of the weak acid to the strong base. Therefore, it varies from 0 to 1 for an acid that releases one proton (monoprotic), from 0 to 2 for a diprotic acid, 0 to 3 for a triprotic acid, etc. In order to calculate equivalents, you would need to know the concentration of the weak acid that you are titrating.

*It is also possible to define equivalents in terms of an HCl if you started the titration with the salt (e.g. NaA), and add HCl. This case the scale is reversed.*

- **The number of NaOH equivalents gives the fraction deprotonated at any given pH on the titration curve.**
- **The number of HCl equivalents gives the fraction protonated.**

**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.

**Buffering range/region:**

\[
\text{PH} = \text{pK}_a \pm 1
\]

**Buffering capacity:** Total moles of a strong acid or base that can be absorbed by a buffer solution and keep the pH within the buffer region. The buffer capacity depends on the pH and the pKa. It is given by:

\[
\text{Capacity} = \Delta \text{equivalents} \times [A^-]
\]

**Example:** What is the buffer capacity of a 0.2 M buffer of a weak acid with a pK_a = 5, assuming a starting pH of 4.5 and the reaction that is buffered consumes protons (pH rises)?

\[
0.6 \text{eq} \times 0.2 \text{M} = 0.12 \text{ M}
\]
Buffers Construction: Need to determine the ratio of \([A^-]\) to \([HA]\) to give desired pH of the solution.

**Typical Problems:**
- \(\text{concentration} \ [A_1], \ [A_1] = [HA] + [A]\) for monoprotic (diprotic \([A_1] = [HA] + [H_2A] + [HA] + [A])\)
- \(\text{volume} V\)
- \(\text{pH} \)
- \(\text{List of weak acids and their pKa values.}\)

**Method - Monoprotic Buffer:**
1. Select a weak acid whose pK_a is within one pH unit of the desired pH. (this applies to all buffers)
2. Determine the fraction protonated and deprotonated at the desired pH, \(f_{HA}\) & \(f_{A^-}\). (need to select the correct pKa for multi-protic buffers).
3. Obtain this ratio of [HA] to [A] by one of the following three methods:
   - i) Mix the indicated concentration of the weak acid and its conjugate base (e.g. sodium salt) to give the desired pH:
     \[
     \begin{align*}
     \text{moles (HA)} &= f_{HA} \times [A_1] \times V \\
     \text{moles (A\textsuperscript{-})} &= f_{A^-} \times [A_1] \times V \\
     \end{align*}
     \]
   - ii) Use \([A_1]\) amount of the acid form of the weak acid and add sufficient strong base (e.g. NaOH) to make the required concentration of \([A]\) to attain the desired pH. You are titrating starting from the left side and converting enough of the fully protonated acid to give the correct amount of the deprotonated acid. The added base converts HA to A\textsuperscript{-}.
     - **The amount of strong base to add is** \(f_{A^-}\) equivalents. \(\text{moles NaOH} = f_{A^-} \times [A_1] \times V\)
     - **This would be added to \([A_1]\) moles of the weak acid.**
   - iii) Use \([A_1]\) amount of the conjugate base form of the weak acid and add sufficient strong acid (e.g. HCl) to make the required concentration of [HA] to attain the desired pH. You are protonating the fully deprotonated acid by just the right amount to give the correct amount of the protonated acid. The added acid converts A\textsuperscript{-} to HA.
     - **The amount of strong acid to add is** \(f_{HA}\) equivalents. \(\text{moles HCl} = f_{HA} \times [A_1] \times V\)
     - **This would be added to \([A_1]\) moles of the weak acid.**

**Example:** Make 1L of 1 M buffer solution at pH 5.0 using either imidazole (pK_a ~ 6), or pyruvate (pK_a ~ 2.5). You have both the protonated and deprotonated species (Na salt) in hand.
1. Which buffer would you use and why?

2. Determine fraction protonated and deprotonated at the desired pH: \(R = 10^{\text{pH} - \text{pK}_a}\)
   \[
   f_{HA} = \frac{1}{1 + R} = \frac{1}{1 + 0.1} \approx 0.9 \\
   f_{A^-} = \frac{R}{1 + R} = \frac{0.1}{1 + 0.1} = 0.1 \\
   \]
3. Since we have both forms (HA), (A) we can use any of the three methods to make the buffer:
   - **method i:**
     \[
     \begin{align*}
     \text{moles (HA)} &= f_{HA} \times [A_1] \times V \\
     \text{moles (A\textsuperscript{-})} &= f_{A^-} \times [A_1] \times V \\
     \end{align*}
     \]
     \[0.9 \times 1M 	imes 1L = 0.9 \text{ moles} \\
     0.1 \times 1M 	imes 1L = 0.1 \text{ moles}\]

4. In practice, for methods ii and iii, the number of moles of NaOH or HCl is not directly measured, rather a pH electrode is used to monitor the pH of the solution, and NaOH or HCl is added until the desired pH is reached.
Polyprotic Buffers:
1. Select pKa that is closest to the desired pH, use that one for your calculations.
2. Calculate \( f_{\text{HA}} \) and \( f_{\text{A}^-} \) using that pKa, and the desired pH.
3. Adjust the pH using either of the three methods:
   i) mix the acid form and the base form of the compound in the ratio of \( f_{\text{HA}} \) to \( f_{\text{A}^-} \). The actual chemicals will depend on the buffer region that was used (see below).
   ii) Start with the fully protonated form and add sufficient base to reach desired pH. The number of equivalents is \( f_{\text{HA}} \) plus additional equivalents to reach the buffer region you used.
   iii) Start with the fully deprotonated form and add sufficient strong acid to reach desired pH. The number of equivalents is \( f_{\text{HA}} \) plus additional equivalents to reach the buffer region you used.

Example: Make 1L of a 0.2M phosphate buffer with a pH = 8.0. pKa values are 2.1, 7.2, and 12.7.
1. Use pKa closest to desired pH.
2. Calculate \( f_{\text{HA}} \) and \( f_{\text{A}^-} \).

3. Select one of the following three methods:
   i) Use chemical forms of "(HA)" and "(A-)" that represent the species present at the pKa you used, in this case. \( \text{NaH}_2\text{PO}_4 = "\text{HA}" \), \( \text{Na}_2\text{HPO}_4 = "\text{A}" \).

\[
\text{moles } \text{NaH}_2\text{PO}_4 = f_{\text{HA}} \times [A^-] \times V \\
\text{moles } \text{Na}_2\text{HPO}_4 = f_{\text{A}^-} \times [A^-] \times V.
\]

ii) Starting from completely protonated form (\( \text{H}_3\text{PO}_4 \)). Add sufficient whole equivalents \( n \) to reach the buffer region you are using, plus and additional \( f_{\text{A}^-} \) to reach the pH with \( n \) that buffer region. In this case:

\[
eq \text{NaOH} = 1 + f_{\text{A}^-}
\]

iii) Starting from completely ionized form (\( \text{Na}_2\text{PO}_4 \)). Add sufficient whole equivalents \( n \) to reach the buffer region you are using, plus and additional \( f_{\text{HA}} \) equivalents of HCl to get to the desired pH in the buffer region. In this case:

\[
eq \text{HCl} = 1 + f_{\text{HA}}
\]
Determining pH:
The overall charge on a molecule as a function of pH can be calculated by summing the contribution from each ionizable group:

i) Identify all ionizable groups on the molecule & their charge when protonated and deprotonated.

ii) Use the known pK_a of each group to determine the fraction protonated (f_{HA}) and deprotonated (f_{A^-}) at the required pH.

iii) Calculate the overall charge by summing the contribution of each group.

Example: What is the net charge on glycine at pH=8?

These calculations are repeated for multiple pH values, to give a plot of charge versus pH:

\[ q_{total} = \sum (f_{HA} \cdot q_{HA} + f_{A^-} \cdot q_{A^-}) \]

- pH = pI, average charge over all molecules in solution is zero
- when pH < pI, q is positive
- when pH > pI, q is negative
Sample buffer problems:

The goal in making a buffer is to generate a solution of a weak acid whose pH doesn't change very much if a strong acid (e.g. HCl) or a strong base (NaOH) is added. The steps in making a buffer are as follows:

1. Select a weak acid which has a pKₐ value within 1 unit of the desired pH of the solution. It does not matter whether it is a monoprotic acid or a polyprotic acid.

2. Sketch a titration curve for the compound that you have chosen. This will help you interpret the number of equivalents needed to make the buffer, especially useful for polyprotic buffers. You may find it useful to draw an additional scale on your titration curve for the reverse titration, adding a strong acid to the fully deprotonated weak acid.

3. Determine the ratio of the protonated weak acid, HA, to the deprotonated form, A⁻, that will give the desired pH. Use the following formula to determine this ratio:

   \[ R = 10^{\frac{pH - pK_a}{1 + R}} \]

   \[ f_{HA} = 1/(1+R) \] \[ f_A = R/(1+R) \]

   The pH of the solution is defined by the problem, use the pKₐ value that is closest to the desired pH.

4. Using the \( f_{HA} \) and \( f_A \) values to determine how to actually generate the buffer. Remember, the only thing that matters is that the final solution has a ratio of HA to A⁻ (\( f_{HA} \) to \( f_A \)) that gives the desired pH. There are three methods of generating the buffer:

   a) mix the amount of the protonated weak acid and its conjugated base to give the desired ratio.

   b) start with the fully protonated form and add \( f_A \) equivalents of base to generate the desired ratio by converting part of the initial HA to A⁻.

   c) start with the fully deprotonated form of the weak acid (e.g. the sodium salt) and add \( f_{HA} \) equivalents of acid to generate the desired ratio of HA to A⁻.

   In the case polyprotic acids and methods b) or c) it may be necessary to add one full equivalent of base or acid to fully titrate or protonate the initial species in order to bring the pH into the buffer region that you are using. Method a) works in all cases, provided the compounds are available.

   Convert from equivalents to moles: Remember that the definition of an equivalent is the ratio of the concentration of the strong base to the concentration of the weak acid:

   \[ eq = [OH^-]/[A^-] \]

   For example, if you make a buffer where the concentration of the weak acid is 0.05 M, then one equivalent of base is 0.05 M. Consequently, if you determine that you need \( Y \) equivalents of strong acid or strong base to generate the buffer, then you will need to add \( Y \times A^- \) to the solution, where \( A^- \) is the concentration of all species of the weak acid that is acting as a buffer, 0.05 M in this example. The units in this case will be moles/L. To obtain the number of moles, multiply by the volume in liters.

Sample Problems — Try to do these problems and then check the solutions on the next page.

1. Make 2 liters of a buffer solution at pH 4.0 using a monoprotic weak acid with a pKₐ of 5.0. The concentration of the weak acid in this solution is 0.01 M.
   a) Show all three methods to make the buffer.
   b) What is the capacity of your buffer, assuming the reaction produces acid?
   c) What is the capacity of your buffer, assuming the reaction consumes acid?

2. You need to make 50 ml of a buffer at a pH = 2.5, the concentration of the buffer should be 0.5 M. You only have succinate to act as your buffer. Succinate is a diprotic acid with pKₐ values of 3 and 6.
   a) Show all three methods to make the buffer.

3. Make 3L of a 0.2 M buffer at pH 7.4 using phosphate buffer. Assume that the pKₐ values for phosphate are 2.2, 7.2, and 12.6. Show all three methods of making the buffer.
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Sample Buffer Problems

Answers:

1. Monoprotic buffer:

   \[ R = 10^{(pH - pK_a)} \]
   \[ f_{HA} = \frac{1}{1 + R} \]
   \[ f_{A^-} = \frac{R}{1 + R} \]

   \[ R = 10^{(4 - 9)} = 0.1 \]
   \[ f_{HA} = \frac{1}{1 + 0.1} = 0.91 \]
   \[ f_{A^-} = \frac{0.1}{1.1} = 0.09 \]

   a) Generating the buffer

   i) Mix 0.91 equivalents of the protonated weak acid with 0.09 equivalents of the sodium salt of the weak acid. To convert from equivalents to concentration, multiply by the concentration of the weak acid. To determine the number of moles, multiply by the volume of the buffer:

   moles HA = \( f_{HA} \times [A^-] \times V = 0.91 \times 0.01 \) moles/L x 2 L = 0.0182 moles HA.

   moles NaA = \( f_{A^-} \times [A^-] \times V = 0.09 \times 0.01 \) moles/L x 2 L = 0.0018 moles NaA.

   ii) If you only had the fully protonated form of the weak acid, you would need to add 0.09 equivalents (= \( f_{HA} \)) of the strong base to generate the correct ratio of HA to A\(^-\) for the weak acid, as indicated in the diagram above. A total of 0.0018 moles of NaOH (0.09 eq x 0.01 moles/L x 2L).

   iii) If you only had the sodium salt of the weak acid, you would need to add 0.91 equivalents (= \( f_{A^-} \)) of the strong acid to generate the correct ratio of HA to A\(^-\) for the weak acid, as indicated in the diagram above. This would be 0.0182 moles of the strong acid (HCl); 0.0182 = 0.91 eq x 0.01 moles/L x 2L.

b) Buffer capacity – acid produced.

   The capacity of this buffer is essentially zero, because any additional acid will move the pH out of the buffer region.

c) Buffer capacity – acid consumed.

   The capacity is 0.91 - 0.1 = 0.81 eq, i.e. the difference in the fraction protonated between the starting pH and the right edge of the buffer region. Since the concentration of the acid is 0.01 moles, a total of 0.81 x 0.01 moles of NaOH could be added before you would leave the buffer region.

2. Diprotic Example: The desired pH falls within the first buffer region, which has a pK\(_a\) = 3. So we will do our calculations assuming that this is a monoprotic buffer with a pK\(_a\) = 3:

   \[ R = \frac{[A^-]}{[HA]} = 10^{(pH - pK_a)} \]
   \[ f_{HA} = \frac{1}{1 + R} \]
   \[ f_{A^-} = \frac{R}{1 + R} \]

   \[ R = 10^{(2.5 - 3.0)} = 0.33 \]
   \[ f_{HA} = 0.75 \]
   \[ f_{A^-} = 0.25 \]

   These calculations are consistent with the pH titration, at pH = 2.5 (< pK\(_a\)) the fraction deprotonated ~ 0.25 (or 0.25 equivalents of NaOH are required to give that pH).

   Method i: moles HA = \( f_{HA} \times [A^-] \times V = 0.75 \times 0.25 \) moles/L x 0.5 L = 0.09375 moles.

   moles AH = \( f_{A^-} \times [A^-] \times V = 0.25 \times 0.25 \) moles/L x 0.5 L = 0.03125 moles.

   Method ii: Using the fully protonated form (HA). This is the same as the monoprotic case since our buffer region is the first ionization – we can ignore the second: eq of NaOH = 0.25

   moles NaOH = \( V \times [A^-] = 0.25 \times 0.5 \times 0.05 \) L = 0.00625 moles.

   This would be added to [A\(^-\)] x V moles of HA = 0.025 moles.
Method iii - using the disodium salt.

\[ \text{eq. of HCl} = 1 + 0.75 = 1.75 \text{ eq} \]

moles of HCl = 1.75 \times [A_1] \times V = 1.75 \times 0.5 \text{ moles/L} \times 0.05 \text{ L} = 0.0437 \text{ moles HCl.} \]

This would be added to [A_1] \times V \text{ moles of Na}_2\text{A} = 0.025 \text{ moles.}

3. Tripotric Example.

Since the desired pH of 7.4 falls within the 2nd buffer region, the correct pK_a value to use in calculating \( f_{\text{HA}} \) and \( f_a \) is the second pK_a or 7.2. Note that in this case "HA" is \( \text{H}_2\text{PO}_4^- \) and "A" is \( \text{HPO}_4^{2-} \).

\[ R = 10^{(7.4-7.2)} = 1.58. \]

\[ f_{\text{HA}} = 1 / (1 + 1.58) = 0.387. \]

\[ f_a = 1 - f_{\text{HA}} = 0.613. \]

Note that the values of \( f_{\text{HA}} \) and \( f_a \) are consistent with the titration curve, since a pH of 7.4 is past the pK_a of 7.2, more than half of the weak acid should be deprotonated at this stage, i.e. \( f_{\text{HA}} \) should be less than 0.5.

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i) At this pH the \( f_{\text{HA}} = 0.387 \) and \( f_a = 0.613 \). Remember - \( \text{NaH}_2\text{PO}_4 \) = "HA" and \( \text{Na}_2\text{HPO}_4 \) = "A".

To convert to moles:
- \( [\text{NaH}_2\text{PO}_4] = 0.387 \times 0.2 \text{ M} \times 3 \text{ L} = 0.232 \text{ moles} \)
- \( [\text{Na}_2\text{HPO}_4] = 0.613 \times 0.2 \text{ M} \times 3 \text{ L} = 0.368 \text{ moles} \)
both of these would be added to 3L of water.

ii) Beginning with the fully protonated form, phosphoric acid (\( \text{H}_3\text{PO}_4 \)). You would have to add one full equivalent of hydroxide to completely remove the first proton (generating \( \text{H}_2\text{PO}_4^- \)), and then an additional 0.613 equivalents of hydroxide (\( = f_a \)) to reach a pH of 7.4. The total number of equivalents of \( \text{NaOH} \) to add is then 1.613. To convert this to moles:

\[ 1.613 \text{eq} \times 0.2 \text{ M} \times 3 \text{ L} = 0.9678 \text{ moles of NaOH (would be added to 3L of 0.2 M phosphoric acid).} \]

iii) Beginning with the fully deprotonated form, sodium phosphate (\( \text{Na}_3\text{PO}_4 \)). You would first have to add one full equivalent of a strong acid (HCl) to fully convert \( \text{PO}_4^{3-} \) to \( \text{HPO}_4^{2-} \). An additional 0.387 equivalents would be required to reach a pH of 7.4, converting part of the \( \text{HPO}_4^{2-} \) to \( \text{H}_2\text{PO}_4^- \) (38.7 % to be exact). Therefore a total of 1.387 equivalents of a strong acid are required. To convert this to moles:

\[ 1.387 \text{eq} \times 0.2 \text{ moles/L} \times 3 \text{ L} = 0.8322 \text{ moles of HCl (would be added to 3L of 0.2M sodium phosphate).} \]