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.

Warm-up: Fraction Protonated versus pH – Sketch the curve for fraction protonated versus pH for a group with a pKₐ=2.

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

Chemical bonding and effects on pKa:

<table>
<thead>
<tr>
<th>Chemical Bonding</th>
<th>pKₐ</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH-H → N-H⁺</td>
<td>10</td>
<td>Easier to break an N-H bond versus an O-H bond, therefore a protonated amine is a stronger acid than an alcohol.</td>
</tr>
<tr>
<td>O-H → O⁻</td>
<td>14</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>H⁺O⁻ → H₂O⁻</td>
<td>4.0</td>
<td>Negative charge delocalized over C=O, lower in energy, therefore a carboxylate is a stronger acid than an alcohol.</td>
</tr>
<tr>
<td>N-O⁻ → N⁻</td>
<td>2.0</td>
<td>Electronegative nitrogen can withdraw some charge from the negatively charged carboxylate, giving a stronger acid than just the COOH.</td>
</tr>
</tbody>
</table>

Boltzmann Distribution: The relative populations \( n_b \) of two states depends on the energy difference between them, \( \Delta E \):

\[ \frac{n_b}{n_a} = e^{\Delta E/kT} \]

Environmental effects on acid strength:
The electrostatic 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=6.0)

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 pKₐ 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?

Zwitterion: a compound that is ionized, but has no net charge.

Isoelectric pH = pI, the pH where the net charge is zero.

Measuring the pKₐ: Titration Curves

Kₐ 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:

pKa Determination (Inflection point): There is an inflection point at the point where the weak acid is ½ deprotonated. Since the two forms of the acid (HA, A⁻) are equal and the pH=pKₐ of the acid.

Equivalence Point: 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.
**Equivalents:** the x-axis can be converted to a scale of equivalents, defined as the ratio of the moles of the strong base to the weak acid. 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.**

**Other ways to measure pKa:** Any method that can give the relative concentration of [HA] and [A] as a function of pH can be used to measure the pKa. One method is NMR, where the chemical shift of atoms may depend on the protonation state. The observed chemical shift is just the weighted average of the chemical shift for the protonated and unprotonated species.

See Lecture 5 Packet: \[ \delta = f_{HA}\delta_{HA} + f_A\delta_A \]

**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:

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. It depends on the concentration of the weak acid, and where the pH is relative to the edges of the buffer region. The more weak acid, the higher the capacity.

Buffers Construction: Need to determine the ratio of $[A^-]$ to $[HA]$ to give desired pH of the solution.

Typical Problems - Monoprotic Buffer:
- concentration $[A_1]$, $[A_1] = [HA] + [A]$
- volume $V$, pH
- List of weak acids and their pKa values.

Method:
1. Select a weak acid whose $pK_a$ is within one pH unit of the desired pH.
2. Determine the fraction protonated and deprotonated at the desired pH, $f_{HA}$ & $f_{A^-}$.
3. Obtain this ratio of $[HA]$ to $[A^-]$ in solution by one of the following methods:
   i) Mix the indicated concentration of the weak acid and its conjugate base (e.g. sodium salt) to give the desired pH:
      moles (HA) = $f_{HA} \times [A_1] \times V$
      moles (A-) = $f_{A^-} \times [A_1] \times V$
   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-.
      The amount of strong base to add is $f_{A^-}$ equivalents.
      moles NaOH = $f_{A^-} \times [A_1] \times V$
      This would be added to $[A_1] \times V$ 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 protonated the fully deprotonated acid by just the right amount to give the correct amount of the protonated acid. The added acid converts A- to HA.
      The amount of strong acid to add is $f_{HA}$ equivalents. moles HCl = $f_{HA} \times [A_1] \times V$
      This would be added to $[A_1] \times V$ moles of the weak acid.

Example: Make 1L of 1 M buffer solution at pH 5.0 using either imidazole (pKa~6), or pyruvate (pKa~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: $R = 10^{(pH-pKa)}$
   \[
   f_{HA} = \frac{1}{1 + R}
   \]
   \[
   f_{A^-} = \frac{R}{1 + R}
   \]
3. Since we have both forms (HA), (A) we can use any of the three methods to make the buffer: