Titration of weak acids
Methods To Prepare Buffer solutions

[Diagram showing methods to prepare buffer solutions, including mixing weak acid with strong base (Method 3), neutralization (Method 2), and combining weak acid and salt (Method 1).]
Titration of amino acids

- Amino acids are weak polyprotic acids.
- Neutral amino acids are (gly, ala, threonine) are treated as diprotic acids.
- Acidic amino acids (glu, asp, ...) are treated as triprotic acids.
- Basic amino acids (lys, arg, his) are treated as triprotic acids.
- $pH_m$ is the pH at which the maximum total number of charges present.
- Glycine can be obtained in three forms:
  a) Glycine hydrochloride
  b) Isoelectric glycine
  c) Sodium glycinate

\[
\begin{align*}
\text{glycine hydrochloride} & \\
\text{glycine hydrochloride} & \\
\end{align*}
\]
Titration of amino acids

Example: Calculate the pH of a 0.1M solution of  a) Glycine hydrochloride , b) Isoelectric glycine , c) Sodium glycinate

\[ K_{a1} = 4.57 \times 10^{-3} \]

a) Glycine hydrochloride is a diprotic acid, the carboxylic group is a much stronger acid than the amino group, the pH of the solution is dependent exclusively by the extent the carboxyl group ionizes.

\[ \text{H}_3\text{N}^-\text{C}_2\text{COOH} \rightleftharpoons \text{H}_3\text{N}^-\text{C}_2\text{COO}^- + \text{H}^+ \]

\[ AA^+ \rightleftharpoons AA^+ \]

Thus, \( y = [\text{H}^+] \) produced, and \( y = [\text{AA}^+] \) produced.

\[ \text{AA}^+ \] remaining at equilibrium = \([\text{AA}^+]\) original - \([\text{AA}^+]\) that ionizes = \(0.1 - y\)

Thus, \( K_{a1} = \frac{(y)(y)}{0.1 - y} = 4.57 \times 10^{-3} \)
Titration of amino acids

Since amount of y (value of y) is stronger than that can be ignored,
Thus $4.57 \times 10^{-4} - 4.57 \times 10^{-3} y = y^2$
$y^2 + 4.57 \times 10^{-3} y - 4.57 \times 10^{-4} = 0.0$

$Y = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$, where $a = 1$, $b = 4.57 \times 10^{-3}$, $c = 4.57 \times 10^{-4}$

Thus $y = [H^+] = 1.92 \times 10^{-2}$.
pH = 1.72.

What is the degree of ionization of glycine hydrochloride in this 0.1M solution?

b) The pH of AA° is the pI (the pI is defined as that pH where the predominant ionic form is AA°, so net charge on the amino acid is zero.)
So $pH = \frac{pK_{a1} + pK_{a2}}{2} = \frac{2.34 + 9.6}{2} = 5.97$

c) Sodium glycinate is a diprotic base,
Both the unionized amino group and the carboxylate ion can accept a proton from water, but since the amino group is a much stronger base than the α-carboxylate group, the pH of the solution depends almost exclusively on the extent to which amino group ionizes.
Titration of amino acids

For the amino group:

\[ K_{b1} = \frac{K_w}{K_{a2}} = \frac{10^{-14}}{10^{-9.6}} = 3.98 \times 10^{-5} \]

For carboxylate group:

\[ K_{b2} = \frac{K_w}{K_{a1}} = \frac{10^{-14}}{10^{-2.34}} = 2.19 \times 10^{-12} \]

\[
\begin{align*}
\text{H}_2\text{N} &- \text{C}_\text{R} - \text{COO}^- + \text{H}_2\text{O} \rightleftharpoons \\
\text{AA}^- & \leftrightarrow \\
\text{H}_3\text{N}^+ &- \text{C}_\text{R} - \text{COO}^- + \text{OH}^- \\
\text{AA}^\circ &
\end{align*}
\]

\[ K_{b1} = \frac{[\text{OH}^-][\text{AA}^\circ]}{[\text{AA}^-]} = \frac{(y)(y)}{0.1 - (y)} \]

Because the concentration of sodium glycinate is much larger than \( K_{b1} \) thus \( y \) can be neglected from the dominator.
Titration of amino acids

\[ 3.98 \times 10^{-5} = \frac{y^2}{0.1}, \text{ so } y^2 = 3.98 \times 10^{-6} \]

\[ y = \sqrt{3.98 \times 10^{-6}} \]

\[ y = 1.99 \times 10^{-3} \text{ M, } [OH^-] = 1.99 \times 10^{-3} \text{ M} \]

\[ [H^+] = \frac{K_w}{[OH^-]} = \frac{1 \times 10^{-14}}{1.99 \times 10^{-3}} = 5 \times 10^{-12} \text{ M} \]

\[ pH = -\log[H^+] = 11.3 \]
Amino acids are titrated in exactly the same manner as diprotic and triprotic weak acids.

Neutral amino acid
Titration Curve of Acidic amino Acid (Glutamate)
Titration Curves of Lysine
Titration Curves of Amino Acids

Information that can be obtained from a titration curve:

1- The number of ionizable groups in that amino acid, which can be detected from the number of titration stages in the curve, (or the number of pKa’s or number of flat zones in the curve).

2- Whether the triprotic amino acid is basic or acidic, that can be detected from the pKapKa2.

If it’s value is closer to the value of pKapKa1 (that of the α-carboxyl group), then it is an acidic amino acid.

If the value of it’s pKa2 is closer to the value of pKapKa3 (that of the α-aminogroup), then it is basic amino acid.

3- The pKa values of the amino acid can be obtained from the curve which is equal to the pH value at the mid-point.

4- The isoelectric point, pI for each amino acid can be obtained from the curve by detecting the point where the amino acid is all in the zwitterion form (net charge = 0.0) the pH at that point is the pI.

Or it can be obtained mathematically from;

\[
pI = \frac{pKapKa1 + pKapKa2}{2} \quad (\text{in the case of a neutral amino acid}).
\]

In the case of triprotic amino acids, the pI is calculated from:

\[
pI = \frac{pKapKa1 + pKapKa2}{2} \quad (\text{in the case of acidic amino acids}).
\]
pI = \frac{pK_{a_2} + pK_{a_3}}{2} \quad \text{in the case of basic amino acids.}

5- You can also determine from the curve the pH values at which the amino acid can act as a buffer. (the pH ranges \pm 1 from the pH value of each midpoint).
How to Obtain a Titration Curves of Amino Acids

No of moles of weak acid or a.a

Calculate the first moles of OH by

\[ A = \frac{\text{no of mole of acid or a.a}}{PKa1} \]

Calculate the second moles of OH added

\[ B = \text{No of moles of weak acid or a.a} + A \]

Calculate the third moles of OH added

\[ C = \text{No of moles of weak acid or a.a} + B \]
How to Obtain a Titration Curves of Amino Acids

Sketch the pH curve for the titration of 100ml of 0.1M Glycine with KOH? Pka1=1.71, Pka2=9.6?

No of moles of a.a = M*V

= 0.1 * 0.1

= 0.01 mole

The first moles of OH by A = 0.01 / 1.71 = 0.005

The second moles of OH added B = 0.01 + 0.005 = 0.015

PI = (Pka1 + pka2) / 2

= 5.66
Plot the titration curve of Aspartic acid it has a volume of 100ml and 0.1M
When titrated with 0.1M KOH? Pka1 = 2.09, Pka2 = 3.86, Pka3 = 9.82?
• Plot the titration curve of Lysine which has a volume of 200ml and 0.3M
  When titrated with 0.1M NaOH? \( \text{Pka}_1 = 2.18, \text{Pka}_2 = 8.95, \text{Pka}_3 = 10.35 \)?