Lab 4: The titration of amino acids

Objective:

Introduction: Alpha amino acids are the building blocks of proteins. Almost all proteins consist of various combinations of the same 20 amino acids. Amino acids are compounds containing both an amine group, \(-\text{NH}_2\), and a carboxylic acid group, \(-\text{COOH}\). In addition there is an "R" group which is different for each amino acid. The symbol "R" is used here to represent a generalized abbreviation for an organic group.

\[
\text{NH}_2 \\
\text{R} \quad \text{C} \quad \text{H} \\
\text{COOH}
\]

In physiological systems where the pH is near neutrality, the amino group of an amino acid will be protonated and the carboxylic acid group will be unprotonated:

\[
\text{NH}_3^+ \\
\text{R} \quad \text{C} \quad \text{H} \\
\text{COO}^-
\]

This is called the zwitterion form.

In strongly acidic solutions the carboxylic acid group will also be protonated, while in strongly basic solutions both the carboxylic acid group and the amino group will be unprotonated.

\[
\text{NH}_3^+ \\
\text{R} \quad \text{C} \quad \text{H} \\
\text{COO}^-
\]

acidic

\[
\text{NH}_2 \\
\text{R} \quad \text{C} \quad \text{H} \\
\text{COO}^-
\]

basic

The acid-base behavior of amino acids is best described by Brønsted theory of acids and bases (see text p. 648). A simple amino acid (that does not have an acid or base group in the "R" group) is a dibasic acid in its fully protonated form; it can donate two protons during its complete titration with a base. The titration with NaOH is a two-stage titration represented below.

\[^{+}\text{NH}_3\text{CHRCOOH} + \text{OH}^- \rightarrow ^{+}\text{NH}_3\text{CHRCOO}^- + \text{H}_2\text{O} \]

\[^{+}\text{NH}_3\text{CHRCOO}^- + \text{OH}^- \rightarrow ^{+}\text{NH}_2\text{CHRCOO}^- + \text{H}_2\text{O} \]
The hydrochloride salt of a simple amino acid contains one mole of HCl for each mole of amino acid such that the amino acid is fully protonated:

\[ +\text{NH}_3\text{CHRCOOH} + \text{Cl}^- \]

The titration curve will be **biphasic** (see diagram below). There will be two separate flat portions (called legs) on the titration curve. The midpoint of the first leg (B) is where the amino acid is half in the acidic form and half in the zwitterion form. The point of inflection (C), occurs when all of the original amino acid is in the zwitterion form. The actual pH at which this occurs is called the isoelectric pH (or isoelectric point), and is given the symbol pI (assuming the "R" group has no charge). In the pH titration of an amino acid with a non-ionizable "R" group, the equivalence point occurs at the pI of the amino acid. At the midpoint of the second leg (D), half the amino acid is in the zwitterion form and half is in the basic form.

![Titration curve diagram](image)

<table>
<thead>
<tr>
<th>Equivalent</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100% +\text{NH}_3\text{CHRCOOH}</td>
</tr>
<tr>
<td>B</td>
<td>50% +\text{NH}_3\text{CHRCOOH} and 50% +\text{NH}_3\text{CHRCOO}^-</td>
</tr>
<tr>
<td>C</td>
<td>100% +\text{NH}_3\text{CHRCOO}^- (First endpoint)</td>
</tr>
<tr>
<td>D</td>
<td>50% +\text{NH}_3\text{CHRCOO}^- and 50% NH\text{H}_2\text{CHRCOO}</td>
</tr>
<tr>
<td>E</td>
<td>100% NH\text{H}_2\text{CHRCOO}^- (Second endpoint)</td>
</tr>
</tbody>
</table>

The apparent pK values for the two dissociation steps may be extrapolated from the midpoints of each step. This can be shown by the **Henderson-Hasselbach equation**:

\[
pH = pK_a + \log \frac{[\text{anion}]}{[\text{acid}]} \]
The $pK_{\text{acid}}$ ($pK$ for the carboxylic acid group) is point (B) where half the acid group has been titrated. Therefore the equation becomes:

$$pH = pK_a$$

In the same way point (D) gives us the $pK_{\text{amine}}$.

In this experiment you will titrate an unknown amino acid and determine its $pI$, $pK_{\text{acid}}$ and $pK_{\text{amine}}$ and compare your values to the $pI$ values from the text.

**Materials**

- 50 mL buret with stopcock
- three 150 mL beakers
- magnetic stirring bar
- three 100 mL beakers
- magnetic stirrer
- 25 mL pipet
- pH meter with electrode
- 10 mL pipet
- pipet bulb
- Kimwipes
- NaOH solution (~ 0.3 M)
- amino acid hydrochloride salt solution A (0.1 M)
- amino acid hydrochloride salt solution B (0.1 M)
- Buffer solutions for calibrating the pH meters

**Procedure**

You will titrate one of the amino acid solutions A or B three times. Since there are half as many pH meters as there are students, you will work in groups of two.

**Titration of the amino acid solution**

1. Soak the pH electrode in distilled water while preparing the amino acid solution sample and setting up the buret.
2. Thoroughly rinse the buret with distilled water. The distilled water rinse should drain evenly from the inside surfaces of the buret and leave no droplets of water behind. Repeat the rinsing procedure until the buret drains cleanly.
3. Obtain approximately 100 mL of NaOH solution. Record the concentration. Rinse and then fill the buret (to the top of the graduated markings) with NaOH solution. Remove any air bubbles (especially from the tip of the buret) and note the starting volume (which may or may not be “0.0 mL”).
4. Obtain approximately 35 mL of one of the amino acid hydrochloride salt solutions; record the letter of the solution. Rinse the 10 mL pipet with the deionized water and then rinse with two small portions of your chosen amino acid solution. Pipet 10 mL of your chosen amino acid solution into a clean 100 mL beaker. Add 25 mL of deionized water (for a total volume in the beaker of 35 mL).
5. Calibrate your pH meter following the instructions provided. The meter will remain calibrated until it is turned off.
6. Place the pH electrode assembly and a magnetic stirring bar into the beaker. Clamp the electrode so that the stirring bar will not hit it as stirring occurs. If the electrode is not properly immersed, the pH reading will be erratic.

7. Titrate the amino acid solution with the NaOH from the buret. This run (called the “dry run”) is done by adding the NaOH at ~1.0 mL intervals (note the exact amount dispensed) until you are just past the first endpoint; in other words, keep adding 1.0 mL increments until the pH rises abruptly (the “C” part of the ideal graph on the previous page. Record all of this information in the “dry run” data table. Note that you can calculate the second endpoint’s theoretical volume of NaOH easily by doubling the volume dispensed in getting to the first endpoint.

8. Refill the buret with NaOH solution. Get another clean dry 150 mL beaker and pipet 10 mL of your chosen amino acid solution into it; add 25 mL of distilled water. Titrate the amino acid solution again (“Titration 1” data table); this time, use ~0.5 mL intervals until just before each endpoint and then dropwise until just after each endpoint. This will be the “real” run that you will graph. Continue until 2.5 equivalents of NaOH have been added or the pH reaches about 12.5.

9. Refill the buret and repeat the titration process (“Titration 2” data table) for a fresh batch of the amino acid hydrochloride salt solution.

Waste disposal
Solutions must be between 5 and 12 pH units before being poured down the sink with water. You can lower the pH by adding hydrochloric acid, or raise it by adding small amounts of solid sodium bicarbonate. The pH meters are set up, so pH paper isn’t necessary.

What your data section should look like:
(In your lab notebook, set up tables that look like the following)

Concentration of NaOH solution ___________________________(don’t forget units!)

Amino acid ____ (insert letter of solution used)

Dry run titration (addition in 1 mL aliquots)

<table>
<thead>
<tr>
<th>mL NaOH added</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Clearly, you will need to make the table with significantly more rows — at least twelve rows)

First endpoint __________ mL
Second endpoint __________ mL (this may need to be calculated, rather than measured.)
Questions

1. I trust that you will be able to write a two-sentence objective given all the information on the previous pages.

2. The amino acids A and B used in this lab are from the following list: glycine, aspartic acid, glutamic acid and lysine. Look up the structure of each of these on page 645 of the text. Identify the R group of each and draw the structural formulae (acidic, basic and zwitterionic) of each as the titration proceeds. Note that some of the amino acids will have three forms and others will have four.

3. Is the completely uncharged form of any amino acid ever seen; in other words, can the uncharged amine group and the uncharged carboxylic acid group ever exist at the same pH? Explain your answer.

4. Prepare a graph of your results, plotting mL of NaOH versus pH. Plot each titration run on a separate sheet of graph paper. Actually, I recommend using Excel for this. Remember to label the graph axes and provide enough tick marks along each axis to be useful; also, give the graph an appropriate title. It is easy to forget these steps when using software; write the information in by hand, if necessary.

5. From a titration curve it is easy to discern the inflection point (pI). This is the point when 1.0 equivalent of base has been added. In this experiment you use 10 mL of 0.1 M amino acid or 1.0 millimoles of amino acid (fully protonated). As stated in the introduction the completely protonated amino acid can donate two protons during the titration. It will take one equivalent (1.0 millimoles) of base to titrate the first proton on the acid group and another equivalent to titrate the proton on the amine group. Find the pI and label it on each graph.
6. Since the $pK_{\text{acid}}$ is the midpoint on the first leg, you can find the $pK_{\text{acid}}$ at 0.5 equivalents of base. Likewise, the $pK_{\text{amine}}$ can be found at 1.5 equivalents of base. Find and label the $pK_{\text{acid}}$ and $pK_{\text{amine}}$ for each graph (if possible).

7. a. average the $pK_{\text{acid}}$ values from the last two graphs.

b. average the $pK_{\text{amine}}$ values from the last two graphs (if possible).

c. average the $pI$ values estimated from the last two graphs.

d. (if possible) calculate the $pI$ by finding the average between the average $pK_{\text{acid}}$ value and the average $pK_{\text{amine}}$ value. This should be a better method of determining the $pI$ of the amino acid. Comment on how close you were by eyeballing the $pI$ value off of the graphs (in other words, compare the value from part c with what you calculated in part d).

8. Identify your amino acid from the following list: glycine, aspartic acid, glutamic acid or lysine. Look up the “true” $pI$ of the amino acid on page 645 of the text, and calculate a percent error between your $pI$ value and the “true” $pI$ value.

9. Comment about how effective this method is at identifying amino acids; specifically, if you did not have a choice of three candidate amino acids, would you have been able to pick yours out from among the twenty possible amino acids? If your $pK_{\text{amine}}$ was impossible to determine, suggest a reason why the “jump” did not show up.

To turn in: photocopy the objective, data tables and answers to the questions. Attach the titration graph.