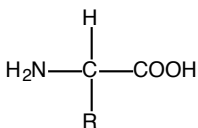
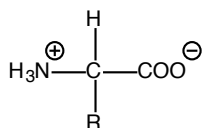


The Titration of Amino Acids

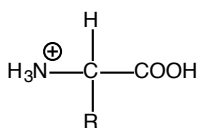
Introduction: Alpha amino acids are the building blocks of proteins. Almost all proteins consist of various combinations of the same 20 amino acids. Amino acids are compounds containing an amine group, $-\text{NH}_2$, and a carboxylic acid group, $-\text{COOH}$. In addition there is an "R" group that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



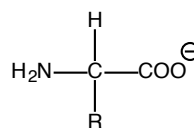
In physiological systems where the pH is near neutrality, the amino group of an amino acid will be protonated and the carboxylic acid group will be deprotonated. This is called the zwitterion form.



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 both be unprotonated.

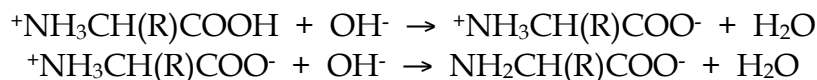


Acidic



Basic

The acid-base behavior of amino acids is best described by the Brønsted-Lowry theory of acids and bases. A simple amino acid (that does not have an acid or base group in the "R" group) is a diprotic 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 by the reactions below.



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.



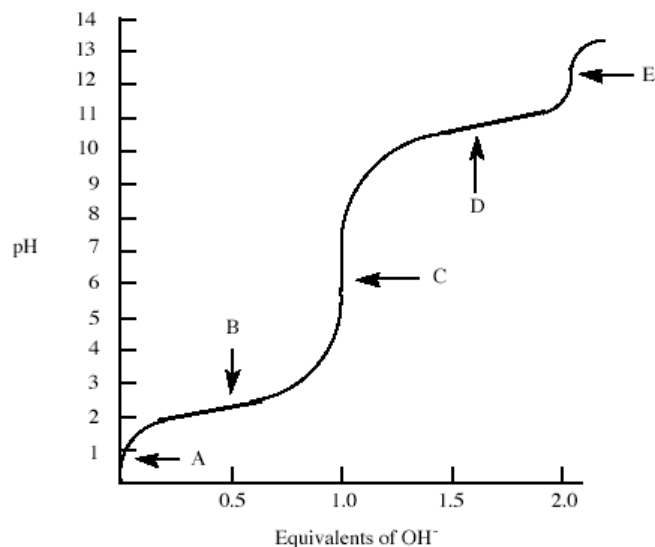
The titration curve will be biphasic (see diagram below). There will be two separate flat portions (called legs) on the titration curve. The midpoint of the first leg (B) is where the amino acid is half in the acidic form and half in the zwitterion form. The point of inflection (C) occurs when all of the original amino acid is in the zwitterions form (assuming the "R" group has no charge). The actual pH at which this occurs is called the isoelectric pH (or isoelectric point), and is given the symbol pI. During 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. 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:

$$\text{pH} = \text{pK}_a + \log\left(\frac{[\text{Base}]}{[\text{Acid}]}\right)$$

The pK_{acid} (pK_a for the carboxylic acid group) is point (B) where half the acid group has been titrated. Therefore the equation becomes:

$$\text{pH} = \text{pK}_a$$

In the same way, point (D) gives us the pK_{amine} . In this experiment you will titrate an unknown amino acid, determine its pI, pK_{acid} and pK_{amine} , and compare your values to literature values.



- A: 100% $^+\text{NH}_3\text{CHRCOOH}$
- B: 50% $^+\text{NH}_3\text{CHRCOOH}$ and 50% $^+\text{NH}_3\text{CHRCOO}^-$
- C: 100% $^+\text{NH}_3\text{CHRCOO}^-$ (First endpoint)
- D: 50% $^+\text{NH}_3\text{CHRCOO}^-$ and 50% $\text{NH}_2\text{CHRCOO}^-$
- E: 100% $\text{NH}_2\text{CHRCOO}^-$ (Second endpoint)

Prelab: Your amino acid will be one of the following: glycine, aspartic acid, lysine, phenylalanine. Answer the following questions on separate paper before class on Wednesday.

1. Look up the structure of each of these on page 667 of the text. Identify the R group (the variable part) 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.

2. 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.

Some of these are diprotic and some are triprotic. When you titrate, you won't know which type you have, so you need to consider all eventualities. The amino acid website I gave you in Activity 3 can help with this, you can see what each curve ought to look like, all on the same graph for comparison. However, remember the curves are idealized, the dilution of the base during titration has not been included. The dilution effect often results in the expected steep region of the last equivalence point being flattened out, so it is not distinguishable from the buffer region preceding it.

3. Go to the tutorial at <http://cti.itc.virginia.edu/~cmg/Demo/titr.html> and click on 3) Compare the titrations of two or more amino acids. Follow the directions to graph the four amino acids of interest. As you add amino acids, the pKa's for each are given below the graph in the same color as for the graph of that amino acid. Prepare a data table listing the pKa's of all four amino acids and their pI's. Calculate pI (the isoelectric point) from the pKa's. You will want to put this data in your lab notebook as well.

4. Clear the graph and redo it with only the triprotic amino acids. Looking at these curves, what do you notice that is odd? You should keep this in mind when performing your titrations, it is why I recommend that you assume your amino acid is triprotic (that is, that you titrate out to three equivalents of base added).

Procedures: You will titrate one of the amino acid solutions A or B three times.

Titration of the amino acid solution

1. Soak the pH electrode in distilled water while preparing the amino acid solution and setting up the buret. Also soak the electrode in distilled water between titrations. Do not hit the calibrate button on the pH meter; the meters are already calibrated for you.

2. Obtain approximately 100 mL of NaOH solution. Record the concentration. Rinse and then fill the buret (to near 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 "0.00 mL").

3. Obtain approximately 35 mL of your assigned amino acid hydrochloride salt solution; record the letter of the solution. Pipet 10.00 mL of your 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).

4. 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.

5. Titrate the amino acid solution with the NaOH from the buret. The first run (called the "dry run") is done by adding the NaOH at ~1 mL intervals (note the exact amount dispensed each time) until you are just *past* the first endpoint; in other words, keep adding 1 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.

6. Refill the buret with NaOH solution. Get another clean dry 150 mL beaker and pipet 10.00 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.

7. Refill the buret and repeat the titration process ("Titration 2" data table) with another sample of the amino acid hydrochloride salt solution.

Waste disposal: This experiment's waste must have its pH adjusted before it can be disposed to the sewer. The lab tech will do that, so we will collect the waste as usual in a large plastic beaker.

Data:

In your lab notebook record the following:

- Concentration of NaOH solution
- Amino acid (A or B) and volume titrated
- Dry run titration
 - Make a table of buret reading, mL NaOH added, and pH reading
 - First endpoint (mL NaOH added)
 - Second endpoint (mL NaOH added; this may need to be calculated, rather than measured)
- Titration 1
 - Make a table of buret reading, mL NaOH added, and pH reading

- Titration 2
 - Make a table of buret reading, mL NaOH added, and pH reading

Analysis:

1. Write a brief objective for this experiment in your own words.
2. Prepare a graph of your results, plotting mL of NaOH added versus pH. Plot each of the two titrations (not the dry 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.
3. Graphically determine the values of pKa's for your amino acid from your titration graphs. Each graph should show the location of the pKa's and the numerical value you determined for each. Remember, pKa's are at half-equivalence points.
4. pI is the pH at the isoelectric point, where the amino acid is in its zwitterion form, which occurs at one of its equivalence points. But pH often changes rapidly at equivalence points, so determining the pI graphically is usually not recommended. Instead, it is determined by averaging the pKa's of the two adjoining buffer regions, which can be determined with reasonable accuracy from a good graph, since they lie in the relatively flat buffer regions. Show the location of the isoelectric point, with the pI you calculated for it on each graph.
5.
 - a) Average the pK_{acid} values from the two graphs.
 - b) Average the pK_{amine} values from the two graphs.
 - c) Average the pK_R values from the two graphs, if your amino acid was triprotic.
 - d) Average the pI values from the two graphs.
6. Identify your amino acid from the following list: glycine, aspartic acid, lysine or phenylalanine, by comparing the pKa's and pI to the values you obtained while doing the prelab assignment. Calculate percent errors for your pKa and pI values using the values from the prelab as true values.

Conclusion: As usual, state your results, their errors, and any problems you encountered and their possible remedies. Also, comment about how effective this method is at identifying amino acids; specifically, if you did not have a choice of four candidate amino acids, would you have been able to pick yours out from among the twenty possible amino acids?