

Exercise 4: Using on-line and printed references for amino acids

As the use of computers and the Internet expands in sciences, you will find many tutorial and educational Java applets. These applets, of course, may be used as sources of information, so long as you trust the authors/maintainers of the site. A good site is maintained by the University of Virginia chemistry department. They have a series of tutorials, and we will use the Java applet pertaining to amino acids, and find out if their information is consistent with other sources.

Get onto a web browser and go to:

<http://cti.itc.virginia.edu/~cmg/Demo/titr.html>

Click on selection 1 ("Observe the titration behavior..."). Read the instructions, and click on Begin the Plot. Click on Alanine and then on Graph. A curved red line should appear on the graph. Finally, click on Show pKa's.

1. If the curved red graph line can be broken up into "flat" sections (i.e., nearly horizontal slope sections) and "steep" sections, in which sections do the pKa's seem to be? (Hint: look at the where the straight lines intersect) In this section of the graph, does the pH of a solution of alanine change much as you go left or right?
2. The x-axis of this graph is labeled as "total protons dissociated". By about how many pH units does the pH of a solution of alanine change when one proton is dissociated from alanine? Is this a significant change?
3. When a proton dissociates from an amino acid, does the pH increase, decrease or stay the same? On the graph, is proton dissociation connected to steep part of the graph or a flat part? How many protons total dissociate from alanine, then? Hint: look at the x-axis and the graph.
4. Compare the behavior of alanine to another amino acid, arginine. Click Clear and then Arginine, then Graph. How many protons can dissociate from arginine?

5. a. Quantify the pKa's. Fill in the table below (notice that you are going to look up **asparagine** now):

Amino acid	pKa1	pKa2	pI
Alanine			
Asparagine			

b. The pI (also called the **isoelectric point**) is the value of the pH in the middle of the steep part of the curve (also called the **inflection point**). It is calculated by averaging the pKa's of the amino acid. Fill in this column of the table.

6. pKa's are a number used to compare the relative ease of dissociation of particular hydrogens (protons) on various molecules. The lower the pKa (especially pKa1 in amino acid), the easier it is to dissociate the proton from the fully protonated molecule. Which amino acid in the table above can dissociate a proton more easily?

(extra credit) Find the amino acid (out of the twenty) that has the "easiest" proton dissociation.

7. a. Click on Back a couple of times to get to the original menu and click on selection 2 ("Analyze the titration behavior..."). Click on Alanine. Click on the carboxylic acid group on the molecule. What pKa value is the acid associated with?

b. Click on Back and click on the amine group on the molecule. What pKa value is the amine associated with? Hah! It's not in the same spot as for the previous question; in fact, it is somewhere on this page and you did look it up in an earlier question!

c. If you began with a fully protonated molecule, which proton is easier to dissociate, the carboxylic acid proton or the amine proton? Why does this make sense (hint: is an amine group acidic or basic)?

The equation shown on this page is a form of the Henderson-Hasselbalch equation. It is used to calculate pKa's, as shown. $[A^-]$ is the concentration of the anion that is left when the proton dissociates from the amine and $[HA^0]$ is the concentration of the undissociated amine.

8. a. Note that the first column of the table on this page shows the **percent dissociation** of the amine. What does this mean? Do the following to find out. Notice that $[A^-]$ and $[HA^0]$ are found to the right side with boxes next to them. Click the box next to $[A^-]$ and type in "1" (which means 1 M concentration). Click in the box next to $[HA^0]$ and type in any other number (you are setting the concentration of the HA; then click Calculate. A value will pop up in the "Calculated pH" field of the table. Does this value match the "Actual pH" given? Probably not. Hit reset and type "1" for $[A^-]$ and a different value for $[HA^0]$; keep doing this until the two pH columns match. What is the value of $[HA^0]$ which makes the pH columns match?

b. With this value, perform this simple calculation: $100 * [A^-] / ([A^-] + [HA^0])$. Does this match the "percent dissociation" column's value? So **describe** what "percent dissociation" is.

c. Finally, notice that one of the lines in the table contains an "actual pH" which is the same as the pKa of the amine. Look at the value of the percent dissociation that leads to this pH. By definition, then, the pKa of a particular proton on a

group in an amino acid is when _____ % of the molecules of that amino acid have dissociated a proton from that group.

9. Click on Back a couple of times, then select Aspartic Acid. Notice that two different carboxylic acid groups are colored. Which carboxylic acid group (the blue or the green) is the proton more easily dissociated from?

There are other parts of the tutorial you may wish to explore.

Now, time for a reality check. Before the Internet, chemists had to rely on published references to look up numbers such as pKa's and pI's. One such reference is the Merck Index, published annually by the Merck and Co., Inc. Another is the CRC Handbook of Physics and Chemistry, published by the Chemical Rubber Company.

10. a. Use either reference to fill in the following table:

Amino acid	pKa1	pKa2	pI
Alanine			
Asparagine			

b. Please **cite** the reference, including the **edition number** and **year** of publication below.

c. How accurate was the Java applet's numbers compared to the "true" values published by the bound references?