

5	92
B	U
10.81	238.029

Acid-Base 4: Identification of an Unknown Amino Acid by Determination of Formula Weight and Acid Dissociation Constants

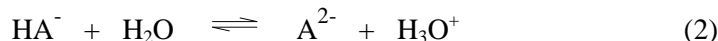
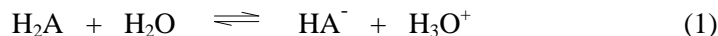
Original, L. Mack and C. Hallen
Amino Acid version, G. Zimmerman (Spring, 2004)

Purpose: Potentiometric titration techniques will be used to determine the molecular mass and acid dissociation constant values for an unknown amino acid. Once these values are determined, the acid will be positively identified.

Introduction: In at least one previous experiment, titrations have been used to probe a known system and return concentration information. In this experiment, potential will be measured in the form of pH values and a graphical analysis of pH with respect to volume of standard base added will be used to determine reaction completion. From the resulting plot, the number of acidic protons, their strengths, as well as the gram molecular masses will be determined. By reference to a table listing the pKa and molar masses of several amino acids, the acid can be identified.

The pH of the solution will be measured using a pH electrode. The electrode has a glass membrane as its contact point with the test solution. When this membrane is placed in contact with a solution containing hydrogen ion, an equilibration occurs inside the membrane depending on the activity of hydrogen ions in solutions on each side of the membrane. The inside solution is trapped thus has a constant activity so any change in voltage depends on the hydrogen ion activity in the test solution. Different pH solutions, therefore, cause different voltages to be recorded by the meter or computer to which this electrode is attached. We can calibrate pH to voltage by using test solutions of known pH.

As we have determined in lecture, a generic weak diprotic acid, H_2A , will dissociate in water according to the reactions:



$$K_{a1} = [HA^-][H_3O^+] / [H_2A] \quad (3)$$

$$K_{a2} = [A^{2-}][H_3O^+] / [HA^-] \quad (4)$$

Note: In this experiment, we are assuming that the K_a 's are far enough apart to allow the reactions to proceed independently. See figure 3.

Since we are collecting pH data, let's convert these K_a expressions into their logarithm form:

$$-\log K_{a1} = -\log ([HA^-][H_3O^+] / [H_2A]) \quad (5)$$

$$-\log K_{a1} = -\log [H_3O^+] - \log ([HA^-] / [H_2A]) \quad (6)$$

and

$$-\log K_{a2} = -\log ([A^{2-}][H_3O^+] / [HA^-]) \quad (7)$$

$$-\log K_{a2} = -\log [H_3O^+] - \log ([A^{2-}] / [HA^-]) \quad (8)$$

Using “pfunctions” (minus the log of) yields

$$pK_{a1} = pH - \log ([HA^-] / [H_2A]) \quad (9)$$

and

$$pK_{a2} = pH - \log ([A^{2-}] / [HA^-]) \quad (10)$$

Examination of equations 9 and 10 yield important information that can be used to identify the diprotic acid. When $([HA^-] / [H_2A])$ equals a value of 1,

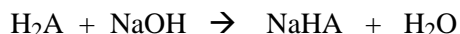
$$pK_{a1} = pH \quad (11)$$

and when $([A^{2-}] / [HA^-])$,

$$pK_{a2} = pH \quad (12)$$

To find the pKa, it is therefore necessary to find the pH at the point where $[HA^-] = [H_2A]$ for pK_{a1} and where $[A^{2-}] = [HA^-]$ for pK_{a2} . To do this, one needs to find both equivalence points using a first derivative plot method, as done previously. Half-way between the beginning of the titration and the first equivalence point is the pH that is equal to pK_{a1} . Half-way between the first equivalence point and the 2nd equivalence point is the pH that is equal to pK_{a2} .

The equivalence point volumes will also be used to find the molecular mass. At the first equivalence point, essentially all of the acid H_2A has been converted into NaHA.



The moles of NaOH used are thus equal to the moles of H_2A initially present:

$$\text{moles base} = \text{moles acid}$$

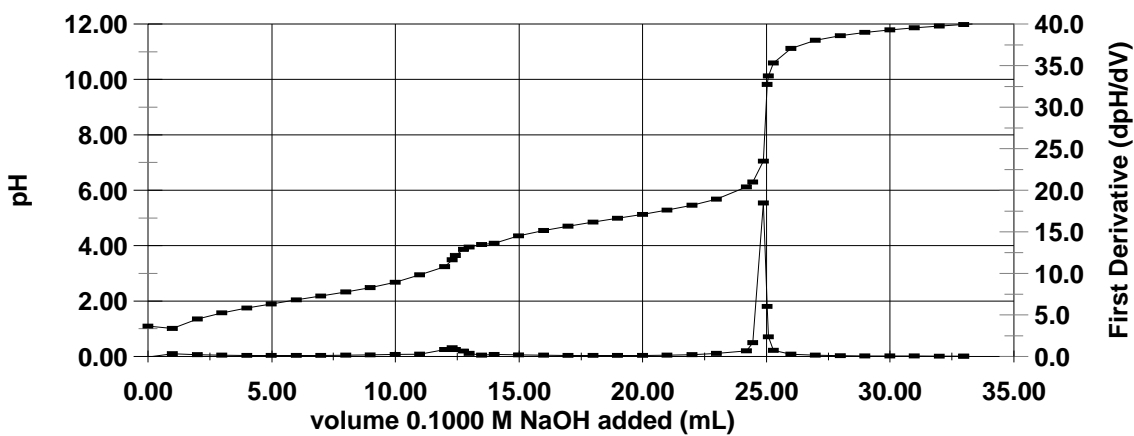
By knowing either the initial mass of acid used, the molar mass of the acid can be calculated. (Just play with the units!)

Example:

A student titrated after dissolving 0.1727 g of an unknown acid with 0.1000 M NaOH. The pK_a values were found by obtaining the volume halfway between 0 and 12.50 (6.25 mL) and halfway between 12.50 and 25.00 (18.75 mL). The data near these points is as follows:

6.00 mL	2.05	18.00 mL	4.85
7.00 mL	2.19	19.00 mL	4.99

Figure1: The titration curve for 0.1727 grams of an unknown acid. The first derivative is shown using the secondary y-axis. Two equivalence point volumes are visible, at 12.50 and 25.00 mL.



Interpolation of the data yields the pK_a 's. Since 6.25 mL is 25% between 6 and 7 mL the pK_a is 25% between 2.05 and 2.19 yielding a value of 2.08 for pK_{a1} . Likewise, pK_{a2} would be 75% between 4.85 and 4.99 yielding a value of 4.96. The molecular weight can be found from the first equivalence point:

$$\begin{aligned} \text{moles base} &= \text{moles acid} = 0.1000 \text{ M} * 0.01250 \text{ L} = 0.001250 \text{ moles} \\ \text{molar mass} &= 0.1727 \text{ g} / 0.001250 \text{ moles} = 138.16 \text{ g/mole} \end{aligned}$$

Using table 1, the best match identifies the acid as anthranilic acid.

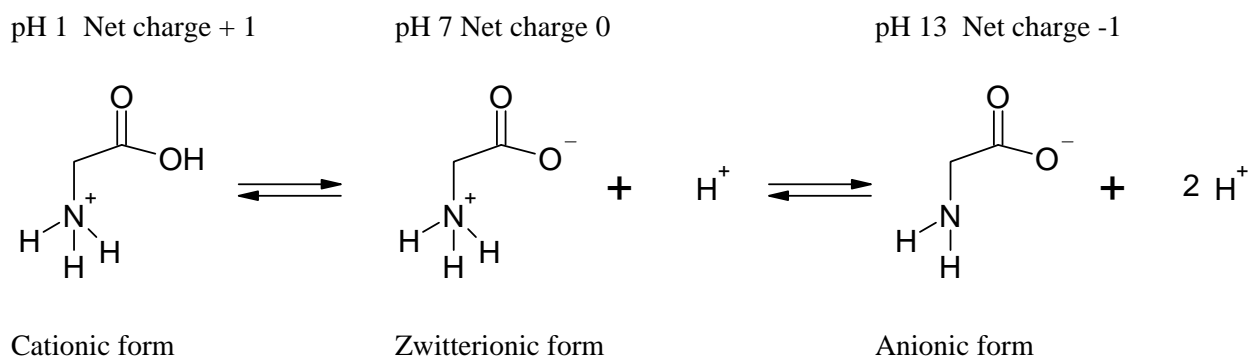
Table 1: Acids on hand - BU Chemistry. (pKa data from a variety of sources)

acid	Molar Mass (Zwitterion)	pKa1	pKa2
Alanine (ala)	89.09	2.34	9.69
Anthranilic (not an amino acid)	138.15	2.08	4.96
Asparagine (asn)	132.12	2.02	8.80
Glutamine (gln)	146.15	2.17	9.13
Glycine (gly)	75.07	2.34	9.60
Leucine (leu)	131.17	2.36	9.68
Proline (pro)	115.13	1.99	10.6
Serine (ser)	105.09	2.21	9.15
Threonine (thr)	119.12	2.63	10.43
Valine (val)	117.15	2.32	9.62

A bit more about amino acids

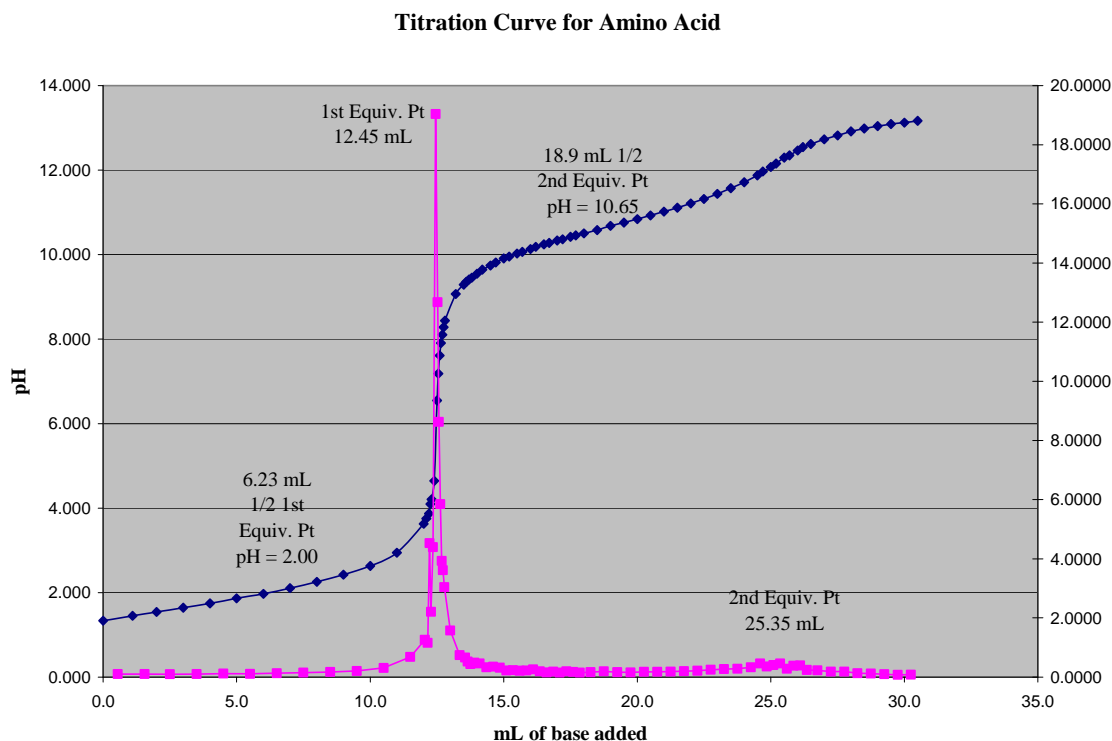
We need to discuss a bit more about amino acids, since they are a little different from the carbonic and oxalic acids covered in class. From a biological point of view, they are the building blocks of life. From a materials science point of view, they are the units(monomers) that build up polymers. A typical amino acid has the form shown in Figure 1 below. There is always a carboxylic acid group (-COOH) and amine group (-NH₂) and an “R” group which are different. The R groups are in fact what make the amino acids different. When the amino acids are placed in a strong acid like HCl, as will be the case for this experiment, the amino acid will be completely protonated and have a net ionic charge of +1. As the base is added in the titration, the proton on the carboxylic acid will be removed first, so that around a pH of 7, the zwitterionic form will exist. At the end of the titration, the amino acid will be completely deprotonated and have a charge of -1.

Figure 1 Forms of an amino acid as the pH changes.



A titration curve therefore looks like Figure 2. The calculations applied earlier now applies.

Figure 2 Titration curve of an amino acid.



Procedure:

Caution: Eye protection must be worn at all times In the laboratory!

Make sure that you record all volume and pH measurements in your notebook as you go. If the computer crashes you will not need to start over.

Obtain either a vial of unknown solid acid from the instructor, or a solution.

Obtain and clean a 50 mL buret. Condition the buret with a standardized sodium hydroxide solution of concentration approximately 0.1 M. Place the buret in a buret holder on a ring stand and fill with the sodium hydroxide solution. Make sure that there are no bubbles in the buret nor in its tip. Record the initial volume reading in your notebook showing two decimal places.

Caution: Sodium hydroxide is a corrosive substance. Be very careful not to get any on your clothes or skin. Clean up spills promptly!

ASK YOUR INSTRUCTOR TO ASSIGN AN UNKNOWN

Pipet 25 mL into a clean 150 mL beaker. Record the mass/volume of acid that is written on the bottle.

Set up the pH electrode as done in the previous experiment. Calibrate the electrode as was done before.

Recheck your initial volume reading on the buret. Titrate the acid solution with sodium hydroxide solution until you are well past the 2nd equivalence point. As before, you want a lot of data points where the pH changes rapidly with volume. By looking at the table 1, we can conclude that if you add base in 1 mL increments up to around a pH of 4, you should be “safe” and not miss the first equivalence point. By observing that the pK_{a2} are between 8.8 and 10.4, you should be able to add 1 mL increments at least up to a pH of about 9 before adding small increments. Read the volume to 0.01 mL and record the value in your notebook.

The data reduction will be performed using Excel.