Amino Acids- Ionic Properties:
Titrations of Amino Acids

**Background Information:**

Begin by reading the section on titrations of amino acids in your biochemistry textbook. There are also several good reference sites on the internet. I have provided two good sites below that you should view prior to performing the lab.

Titrations general information:
Titrations of amino acids:

**Pre-Laboratory Questions** (completed in notebook prior to class):

1. Sketch a titration curve for the amino acid glycine.
2. Sketch a titration curve for the amino acid aspartic acid.
3. Explain the differences in the curves for 1 and 2 (don't describe, explain).
4. Discuss potential sources of error in this experiment, more than one please.

**In Lab Concept Outline:**

1. Acid/base chemistry of amino acids
2. Titrations
3. Experimental determination of pKa values
4. Identification of an unknown amino acid

**Experimental Procedure:**

You will be working in groups of two for this experiment. Each group will choose one unknown amino acid solution to titrate both with acid and with base. We will be using standardized HCl and NaOH solutions (be sure to note the exact molarity of these solutions in your notebook). In addition to titrating the amino acid solution, each group must also titrate a water blank with both the acid and the base solutions. Each person in the group should perform at least one part of the experiment; all data obtained by the group needs to be recorded by each member in the laboratory notebook. Remember to record the unknown number and the names of all group members.

**Methods:**

I. Standardize pH meters: Standardize (calibrate) the pH meter with the pH 4.0 and pH 7.0 standard buffer solutions.

II. Amino Acid Titration
1. Weigh out about 400 mg of an unknown amino acid. Dissolve the solid in 40 mL of distilled water, then bring the total volume to 50 mL with distilled water and place the solution into a beaker.

2. Place a stir bar in the beaker, and mix the solution on the stir plate.

3. Standardize the pH meter with pH 4 standard buffer.
4. Carefully place the pH probe into the amino acid solution, so that the probe is far enough into the solution, but not touching the stir bar or beaker.

5. Begin titrating the solution with the standard HCl solution by adding 0.1 ml aliquots. It is essential to know the exact volume of acid added, so pipet carefully, noting the number of significant figures that will be dictated by the precision of the pipet used. After each addition of 0.1 ml, record the volume added and measure and record the pH of the solution, after it stabilizes.

6. Continue titrating until the pH drops to 1.5.

7. Repeat steps 1-6, only this time, calibrate the pH meter with the pH 10 standard, and titrate with the calibrated NaOH solution until the pH is approximately 12.0. Be sure to start with a fresh amino acid solution.

III. Water Blank Titrations.

1. Place 100 mL of distilled water into a beaker.

2. Place a stir bar in the beaker, and mix solution on the stir plate.

3. Standardize the pH meter with pH 4 standard buffer.

4. Carefully place the pH probe into the water, so that the probe is far enough into the solution, but not touching the stir bar or beaker.

5. Begin titrating the solution with the standard HCl solution by adding 0.1 ml aliquots. It is essential to know the exact volume of acid added, so pipet carefully, noting the number of significant figures that will be dictated by the precision of the pipet used. After each addition of 0.1 ml, record the volume added and measure and record the pH of the solution, after it stabilizes.

6. Continue titrating until the pH drops to 1.5.

7. Repeat steps 1-6, only this time, calibrate the pH meter with the pH 10 standard, and titrate with the calibrated NaOH solution until the pH is approximately 12.0. Be sure to start with 100 mL of fresh distilled water in a beaker.

**Data Manipulation:**

1. Prepare a table of moles of HCl and NaOH added during titration and the observed pH value for the unknown amino acid and the water blank.

2. Subtract the water blank titrant volume from the curve.

3. Plot corrected moles of HCl or NaOH added vs. pH for the amino acid (titration curve).

4. From the plot, determine the number and types of titratable groups for the unknown amino acid.

5. Determine the experimental pKa values for each titratable group from the plot.

6. Determine the molecular weight of your unknown amino acid from data obtained from your plot.

7. What is the identity of your unknown amino acid? Justify your conclusion by comparing the observed molecular weight and pKa values to those for all of the amino acid listed in Table 1.
**Post-Laboratory Questions:**

1. For each functional group of your deduced amino acid, write the ionization reaction; indicate the pH where conjugate acid and base pairs are equal in concentration.

2. Why was it necessary to titrate a water blank?

3. Is the true titration curve of leucyl valine the same as that of glycine? Explain your answer.

4. Calculate the isoelectric point of a) His and b) Asp. Where would you find the isoelectric point on a titration curve?

**Laboratory Report:**

This will not be a full laboratory report. You will need to turn in your plots and the results from the data manipulation section. You will also need to turn in the post-lab questions on a separate sheet of paper. Be sure to include the number of your amino acid and the names of your partners for this experiment in your report. Be sure to include titles, numbers, and legends for your plots. As with any laboratory report, write in full sentences and paragraph format, third person passive voice.

**Table 1. Molecular Weights and pKₐ Values for the Amino Acids.**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>MW (g/mole)</th>
<th>pKa (α-COOH)</th>
<th>pKa (α-amino group)</th>
<th>pKa (side chain)</th>
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</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>75</td>
<td>2.35</td>
<td>9.78</td>
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<tr>
<td>Alanine</td>
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<td>2.35</td>
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<td>Serine</td>
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<td>Proline</td>
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