Histidine is a common amino acid found in many proteins and other natural sources. There are four different states of protonation of histidine as illustrated below.

The four species may be designated as $H_3A^{2+}$, $H_2A^+$, HA, and $A^-$. Note that HA and $H_2A^+$ are zwitterions. The three acid dissociation constants are $K_1 = 2 \times 10^{-2}$ ($pK_1 = 1.7$), $K_2 = 9.5 \times 10^{-7}$ ($pK_2 = 6.02$), and $K_3 = 8.3 \times 10^{-10}$ ($pK_3 = 9.08$), all at $\mu = 0.1$.

A solution of histidine will contain these four species in amounts that depend upon the pH of the solution. There are only one or two dominant species present at a particular pH. Below pH = 1.7 ($pK_{a1}$) $H_3A^{2+}$ is the most abundant species. At pH = 1.7 there is an equal amount of $H_3A^{2+}$ and $H_2A^+$. At pH = 6.02 there is equal amounts of $H_2A^+$ and HA. At a pH $\approx (1.7+6.0)/2 = 3.9$, 98% is in the $H_2A^+$ form. At pH = 9.08 there is equal amounts of HA and $A^-$. At a pH $\approx (9.0+6.0)/2 = 7.5$, 98% is in the HA form. $A^-$ is the dominant species above pH = 9.1.
The titration curve below shows the simulated titration of 25 mL of a 0.1 M \( \text{H}_3\text{A}^{2+} \) solution (prepared by dissolving 0.1 mol of a 2HCl·HA salt in 1 L of water solution) with 0.1 M NaOH. You can see there are three discernable endpoints. The first one comes at a \( V_1^* \) of 25 mL at a pH of about 3.9. The second equivalence point comes at \( V_2^* \) of 50 mL at a pH of about 7.6. The third equivalence point comes at \( V_3^* \) of 75 mL at a pH of about 11.0.

If you were to titrate a 0.1 M solution Histidine (HA) with your standardized NaOH solution and follow it with a pH meter, the titration curve would reveal a starting pH at 7.8 and one equivalence point at about a pH of 11. In other words you would be starting at the 50 mL mark in the \( \text{H}_3\text{A}^{2+} \) titration curve above. This equivalence point would correspond corresponds to the point where all of the HA has been converted to A\(^-\).

If you were to titrate a 0.1 M solution Histidine (HA) with your standardized HCl solution and follow it with a pH meter, the titration curve would reveal a starting pH at 7.8 and one equivalence point at about a pH of 3.8. This equivalence point would correspond corresponds to the point where all of the HA has been converted to \( \text{H}_2\text{A}^+ \). A second equivalence point corresponding to the point at which all of the \( \text{H}_2\text{A}^+ \) has been converted to \( \text{H}_3\text{A}^{2+} \) is not obtainable in aqueous solution because \( \text{H}_3\text{A}^{2+} \) is such a strong weak acid. In other words, in aqueous solutions \( \text{H}_3\text{A}^{2+} \) is always significantly dissociated to \( \text{H}_3\text{O}^+ \) and \( \text{H}_2\text{A}^- \).

In this experiment you will prepare an HCl solution with an approximate concentration of 0.1 M. You will standardize this solution using you standardized NaOH solution from last week. Then you will analyze an unknown histidine at an approximate concentration of 0.1 M. Using a pH meter to follow the titration, you will titrate this histidine solution with your standardized NaOH and then again with your standardized HCl.
**First and second derivative titration curves**

For the titration of dilute or weak acids and bases, including most polyprotic systems, it is often necessary to use a first or second derivative plot to accurately locate the equivalence points. A first derivative titration curve plots $\Delta \text{pH}/\Delta V$ vs. $V_{\text{ave}}$. A second derivative curve plots $\Delta (\Delta \text{pH}/\Delta V)/\Delta V$ vs. $V_{\text{ave}}$. See pg 237 of Harris for an example of calculating these quantities with your data, and examples of how to used the fist and second derivative plots to locate the endpoints. We will prepare all three types of titration curves in this experiment.

**IN THE LAB**

**Preparation of HCl solution**

Add about 200 mL of freshly boiled distilled water to a 1 L polyethylene container. Add approximately 8 mL of concentrated HCl. Screw the lid on the bottle and shake the solution a little. Now fill the bottle to the rim with more freshly boiled distilled water. Screw the lid on the bottle and mix thoroughly. Label the container with your name and “HCl”.

**Standardizing your HCl solution**

Pipet 20.00 mL of your HCl solution into each of three 250 mL Erlenmeyer Flasks. Add three drops of phenothalein indicator. Titrate each of three five samples to the phenolthalein endpoint with your standard NaOH solution.

**The Histidine accident**

An unknown quantity of an HCl or NaOH solution was “mistakenly” added to a 2.000 L of a 0.1242 M histidine solution. The goal of this experiment is to determine the number of moles, volume and concentration of the HCl or NaOH that was added to the histidine standard.

Take about 80-100 mL of the unknown histidine solution that resulted from the “mistake”. Pipette 25.00 mL to each of two 250 mL Erlenmeyer Flasks.

**Calibrating the pH meter**

Set up the buret, pH meter, magnetic stirrer on a ring stand such that you will be able to monitor the pH of a continuously stirred solution during the course of the titration. Be sure not to stir too vigorously. Calibrate the pH meter with pH 7 and pH 10 buffer. Rinse the electrode with the distilled water.
**Titrating the Histidine solution with NaOH**
Titrating one of the histidine solutions with your standardized NaOH, taking an initial pH reading, followed by reading every 0.1 pH unit apart. Wait at least 30 seconds between each addition of NaOH for the solution to come to equilibrium. Continue with the titration until you reach a pH of about 12.3-12.7.

**Titrating the Histidine solution with HCl**
Revitalize the pH meter using buffer 7 and 4. Titrating the other histidine solution with your standardized HCl, taking an initial pH reading, followed by reading every 0.1 pH unit apart. Wait at least 30 seconds between each addition of NaOH for the solution to come to equilibrium. Continue with the titration until you reach a pH of about 1.5-1.7.

**Questions:**

1. For the HCl standardization, calculate the mean V* and its 95% CL.

2. Calculate the average [HCl] for your solution.

3. Propagate the errors in V*, and the glassware (pipet, buret, ect.) and report an error in the [HCl].

4. Construct a titration plot V vs. pH for the histidine titration with NaOH. Also construct a first and second derivative plots. Use the second derivative plot to locate the endpoint(s).

5. Construct a titration plot V vs. pH for the histidine titration with HCl. Also construct a first and second derivative plots. Use the second derivative plot to locate the endpoint(s).

6. From the data calculate the concentrations for the two most abundant species in the unknown histidine solution (ie. HA and H₂A⁺ or HA and A⁻).

7. Calculate the volume and concentration of HCl solution that was “mistakenly” added to a 2.000 L of a 0.1242 M histidine solution to produce your unknown histidine solution.
Note to Instructor:

1) Add at least 200 mL of a dilute (0.1M) solution of HCl.

2) Be sure the water used is clean. Water from the still in P.Chem lab seem to contain significant amount of an unknown weak base, because the data from the NaOH titration did not match the data from the HCl titration