Experiment No. 6

Determination of the pI of an Amino Acid

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 both an amine group, $-NH_2$, and a carboxylic acid group, -COOH. In addition there is an "R" group which is different for each amino acid.

$$^{+}NH_3CHRCOOH + OH^{-} \rightarrow {}^{+}NH_3CHRCOO^{-} + H_2O$$

$$^{+}NH_3CHRCOO^{-} + OH^{-} \rightarrow NH_2CHRCOO^{-} + H_2O$$

In this experiment the titration of the amino acid provided will be carried out in two ways. Method I will involve titration with NaOH alone and Method II will involve titration with HCl and NaOH.

You will determine the pK values and from the information obtained determine the pI of the amino acid given.

Standardization of NaOH (~1 N)

Pipette 25 ml of standard oxalic acid solution into a clean dry conical flask, add 2 drops of phenolphthalein indicator solution, and titrate with NaOH solution to the pink endpoint.

Standardization of HCl solution (~1 N)

Pipette exactly 5.00 ml of the HCl solution into a clean conical flask, add 2 drops of phenolphthalein indicator solution, and titrate to the pink endpoint with standard NaOH.

Preparation of amino acid solution: Prepare 100 ml of a 0.1 M solution of the amino acid considering an approximate molecular weight 110.

Before beginning, calibrate the pH meter using standard buffer solutions of pH 4 and pH 7, or pH 7 and pH 10.

Method I:

Protonation of the amino acid carboxylate group

Transfer exactly 10 ml of the amino acid solution to a clean beaker. Calculate the number of moles of amino acid in the beaker. Calculate the volume of standard HCl needed to provide this same number of moles of HCl. Add this volume of HCl solution to the beaker using a graduated burette. The amino acid carboxylate group should now be protonated.

Amino acid titration

Fill a burette with standard NaOH solution. Rinse and dry the pH electrode and submerge it in the solution containing protonated amino acid.

RECORD THE INITIAL pH OF THE SOLUTION.

Initiate the pH titration by adding 0.5 ml of NaOH solution, stirring the solution thoroughly, and reading the pH. After each pH reading, record the total added volume of NaOH and the pH. Continue adding NaOH 0.50 ml at a time until the total added volume is about 80% of the total required to titrate the -COOH proton. At this point, add NaOH in smaller increments of first 0.20, then 0.10 ml. Continue adding NaOH incrementally until you are past the -COOH equivalence point (pH is approximately 8 or greater). Go back to adding NaOH 0.5 ml at a time until you have added 80% of the amount required to titrate the $-NH_3+$ proton. At this point, add smaller increments as above until you are past the equivalence point (pH is approximately 12).

Repeat the titration at least once.

Method II:

Transfer exactly 25 ml of a 0.1 M amino acid solution to a clean, 100ml beaker. Place a thoroughly rinsed teflon-coated stirring bar into the beaker and place the beaker on a magnetic stirrer. Insert the rinsed electrode of the pH meter into the solution and **record the starting pH**. Obtain ~50 ml of 1 N HCl. With the stirrer turned on, add 0.2 ml HCl using an appropriate pipettor, wait for the pH value to stabilize on the pH meter and record the pH. Again add 0.2 ml HCl while stirring, record the pH and so on. A graph of pH versus milliliters HCl added should be made during the experiment as well as recording the data in tabular form. Continue the titration until the solution reaches a pH of 1.5. Remove the electrode from the solution, wash, and insert it into a beaker of distilled water. Rinse the electrode.

Transfer exactly 25 ml of the same amino acid solution into a clean, 100 ml beaker. Place a teflon coated stirring bar into the beaker and place the beaker on the magnetic stirrer. Insert the rinsed electrode of the pH meter into the solution and **record the starting pH**. Obtain ~50 ml of 1N NaOH. With the stirrer turned on, add 0.2 ml NaOH using an appropriate pipettor, wait for the pH value to stabilize on the pH meter and record the pH. Again add 0.2 ml NaOH while stirring, record the pH and so on. A graph of pH versus milliliters NaOH added should be made during the experiment as well as recording the data in tabular form. Continue the titration until the solution reaches a pH of 12.0. Remove the electrode from the solution, wash and insert it into a beaker of distilled water.

Plot a graph for each case and determine the pI of the amino acid provided.