THE TITRIMETRIC DETERMINATION OF THE CONCENTRATION AND ACID DISSOCIATION CONSTANTS OF AN UNKNOWN AMINO ACID

Topics for Study: Volumetric analysis, primary standards, secondary standard solution, amino acids, equilibrium constants, titration curves, use of the pH meter. Technique videos on the use of a burette and pipet.

Glossary: Zwitterion, equivalence point

TECHNIQUES

In this assignment, you will use the following procedures:

Manipulative Skills
- use an analytical balance
- use a volumetric pipet
- use a pH meter
- perform a titration
- use a spreadsheet

Theoretical Skills
- plot data using a spreadsheet
- determine the pK_a-values of an amino acid
- calculate the concentration of an amino acid solution
- calculate pH-values to generate a titration graph

SAFETY

Always wear safety glasses or goggles, a flame-resistant lab coat (which is not the same like a cotton lab-coat), clothing that covers your legs, and closed-toe shoes. Wear gloves when handling the concentrated NaOH solution. Neutralize all excess base or acid solutions by adding citric acid until the pH is between 4 and 10 before pouring the solution down the drain.

INTRODUCTION

The multitudes of proteins that exist in living matter are among the most complicated molecules known. Proteins perform thousands of functions that are essential for the continuation of life. Extensive ongoing research around the world is investigating these processes and the correlation of protein function with molecular structure. In fact, determining the molecular structure itself is an enormous challenge and was first accomplished by F. Sanger in 1955, who received the Nobel Prize in Chemistry in 1958 for this work. More recently, spectroscopic methods such as multi-dimensional NMR and X-ray crystallography complemented by sophisticated molecular modeling programs carry the brunt of the work.

Although the molecular structures of proteins are complicated, their building blocks, the twenty standard amino acids are not (Table 1). Proteins are often classified in terms of the polarity of the different side chains, which contribute to the nature of the proteins. The differing side chains determine the various properties of the amino acids. The common components in amino acids are the acid (-COOH) and amine (-NH_2) groups. This assignment deals with the commonality, the fundamental acidic (and basic) properties of the amino acids.
Nature of R-group | Amino acid
--- | ---
Nonpolar | glycine (R=H), alanine (R=CH₃), valine (R=CH(CH₃)₂), leucine, isoleucine, proline, phenylalanine (R=PhCH₂), tryptophan, methionine (R=CH₂CH₂SCH₃)
Polar | serine (R=CH₂OH), threonine, asparagine, glutamine (R=CH₂CH₂CONH₂)
Acidic | aspartic acid (R=CH₂COOH), glutamic acid, cysteine, tyrosine (R=CH₂CH₂OH)
Basic | lysine (R=CH₃CH₂CH₂NH₂), arginine, histidine (R=CH₂C₃H₇N₂)

Table 1: Classification of the Amino Acids

In this assignment, you will determine two important properties of an unknown amino acid solution: its concentration in solution and its pKₐ-values. Although the techniques and procedures you will use apply to the analysis of any diprotic acid, you are likely to encounter the acidity of amino acids more frequently in molecular life science than any other type of compound. While the R groups may contain acidic or basic groups, much of the chemistry of the amino acids results from the amine group, -NH₂, and the acid group, -COOH and their relative strengths represented by the pKₐ-values.

![Figure 1: Formulas for ammonia, acetic acid, and an amino acid](image)

Amino Acid Chemistry

If an amino acid is dissolved in water, some dissociation of the acid group occurs as well as some hydrolysis of the amine group. This formally resembles a transfer of the proton from the acid to the base to leave a "dipolar ion" known as a zwitterion. The proximity of the acid hydrogen to the lone pair of electrons on the amine group to allow this transfer is more easily seen in the space-filling model (Figure 2b) than in the ball-and-stick model (Figure 2a).

![Figure 2: Ball-and-stick and space-filling models of the amino acid glycine.](image)

The zwitterion form can also be used to explain the abnormally high melting point of many amino acids (i.e., glycine: 233 °C, alanine: 258 °C, valine: 298 °C) and their high polarity as indicated by the log Kₐw-value (i.e., glycine: -3.21, alanine: -2.86, valine: -2.26).
Extensive studies on amino acids indicate that, in solution, the zwitterion is the predominant species of the neutral form of the amino acid. However, the relative proportions of the neutral amino acid ion (HA\(^{+/-}\)), and the positively (H\(_2\)A\(^+\)) and negatively charged ions, (A\(^-\)) that exist in a solution are determined by the total amino acid concentration in the solution and the pH of the solution (Figure 3).

![Chemical structures and reactions](image)

**Figure 3**: The reactions of an amino acid in water.

If the hydrolysis reaction of Figure 3 is now rewritten as an acid dissociation reaction,

\[
R\text{C} \text{O} \text{H}_3 \text{NH}_3^+ + \text{H}_2\text{O} \rightleftharpoons R\text{C} \text{O} \text{H}_3 \text{NH}_3^+ + \text{H}_2\text{A}^+ + \text{H}_3\text{O}^+
\]

then the mass-action equation becomes

\[
K_{a1} = \frac{[\text{H}_3\text{O}^+][\text{HA}^{+/-}]}{[\text{H}_2\text{A}^+]}
\]
which in logarithmic form can be rewritten as

$$pK_{a1} = \text{pH} - \log \frac{[\text{HA}^+/\text{HA}^-]}{[\text{H}_2\text{A}^+]}$$  \hspace{1cm} (3)

or

$$\text{pH} = pK_{a1} + \log \frac{[\text{HA}^+/\text{HA}^-]}{[\text{H}_2\text{A}^+]}$$  \hspace{1cm} (4)

The acid dissociation reaction of the neutral zwitterion,

$$\begin{array}{c}
\text{HA}^{+/\text{HA}^-} + \text{H}_2\text{O} \\ \rightleftharpoons \\
\text{H}_3\text{O}^+ \text{A}^- \text{H}_2\text{A}^{+/\text{HA}^-}
\end{array}$$  \hspace{1cm} (5)

has a mass action expression of

$$K_{a2} = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}^+/\text{HA}^-]}$$  \hspace{1cm} (6)

In strongly acidic solution, the equilibrium will shift to favor $\text{H}_2\text{A}^+$ as the predominant form of the amino acid; in strongly basic solutions, $\text{A}^-$ will be the major species present. In between there is a range of pH values over which two or three forms of the amino acid have appreciable concentrations.

**Figure 4:** Titration of 25.0 mL of a 0.100 M glycine in the $\text{H}_2\text{A}^+$ form with 0.100 M NaOH
The concentration and dissociation constants of amino acids can be determined by monitoring the pH of the solution with a pH meter during the titration of aliquots of the amino acid with a strong acid or a strong base. If the amino acid exists in the $\text{H}_2\text{A}^+$ form initially, the titration with a strong acid and a strong base will lead to the formation of the neutral zwitterion form ($\text{HA}^{+/−}$) first before yielding the anionic form ($\text{A}^−$). The titration curve will show two buffer regions and two inflection points indicating the equivalence points in the titration. The titration curve for a 25.0-mL aliquot of the protonated form of 0.100 M glycine ($\text{H}_2\text{A}^+$) with 50.0 mL of 0.100 M HCl solution is shown in Figure 4. Note that the change around the second equivalence point is much smaller than the first one.

The same graph would be generated by combining the titration data obtained by titrating a 25.0-mL aliquot of the zwitterion form, ($\text{HA}^{+/−}$) with 25.0 mL of 0.100 M NaOH and another 25.0-mL aliquot with 0.100 M HCl.

For the reaction with HCl,

\[
\begin{align*}
\text{R-CO-NH}_3^+ \quad & \text{H}_2\text{O}^+ \quad \leftrightarrow \\
\text{H}_2\text{A}^+ \quad & \text{OH}^- \\
\end{align*}
\]

and at this half-neutralization point in the titration shown by the lower arrow in Figure 4 [$\text{HA}^{+/−}$] = [$\text{H}_2\text{A}^+$] and the mass action expression, Equation (2), reduces to

\[
K_{a1} = [\text{H}_3\text{O}^+]_1 \text{ or } pK_{a1} = p\text{H}_1
\]

Similarly, for the reaction with hydroxide,

\[
\begin{align*}
\text{R-CO-NH}_3^+ \quad & \text{OH}^- \quad \leftrightarrow \\
\text{A}^- \quad & \text{H}_2\text{O} \\
\end{align*}
\]

at the half-neutralization point shown by the upper arrow in Figure 4, [$\text{HA}^{+/−}$] = [$\text{A}^−$] and Equation 6 reduces to

\[
K_{a2} = [\text{H}_3\text{O}^+]_2 \text{ or } pK_{a2} = p\text{H}_2
\]

Whether or not the amino acid to be titrated is in a neutral or A charged form, once the titration curve has been obtained, the $pK_a$-values can be determined from the graph in the manner outlined above. The difference in volume between the two equivalence points can be used to determine the concentration of the amino acid.
THEORETICAL AND PRACTICAL CONCEPTS

By the end of this assignment, you should be able to

1. Write equations for the reaction of the zwitterion of an amino acid with acid and with base.

2. Calculate the concentration of an amino acid solution from titration data and explain why the concentration can be determined when the amino acid exists in multiple forms at the beginning of the titration. For example, calculate the concentration of the solution if a student titrated 10.00-mL aliquots of her unknown amino acid solution (containing both HA\(^+\) and H\(_2\)A\(^+\) forms of the amino acid) with standard 0.1527 M NaOH. She determined that inflection points in the graphs occurred after 4.27 mL and 19.85 mL of the base were added.

3. Calculate the pH of a polyprotic acid given the pK\(_a\)-values and the volumes and concentrations of acid and base reagents placed in solution.

4. Sketch titration curves showing the key points for the following reactions and be able to explain your reasoning showing necessary calculations (pre-lab for meeting 3).
   a. A 10.00-mL aliquot of 0.100 M maleic acid, H\(_2\)M, titrated with 0.100 M NaOH.
      Acid Dissociation Constants for maleic acid: pK\(_{a1}\) = 1.91; pK\(_{a2}\) = 6.07
   b. A 10.00-mL aliquot of 0.0500 M Na\(_3\)PO\(_4\) titrated with 0.100 M HCl.
      Acid Dissociation Constants for H\(_3\)A: pK\(_{a1}\) = 2.15; pK\(_{a2}\) = 7.20; pK\(_{a3}\) = 12.35
   c. Aliquots of 10.00-mL of 0.100 M NaHSO\(_3\) titrated with 0.100 M HCl or 0.100 M NaOH. (Combine the data from the two titrations into one titration curve)
      Acid Dissociation Constants for Sulfurous acid: pK\(_{a1}\) = 1.85; pK\(_{a2}\) = 7.20
   d. Aliquots of 10.00-mL of a solution that is 0.150 M in NaHCO\(_3\) and 0.850 M in Na\(_2\)CO\(_3\) titrated with 0.100 M HCl and 0.100 M NaOH.
      Acid Dissociation Constants for Carbonic acid: pK\(_{a1}\) = 6.37; pK\(_{a2}\) = 10.32

EXPERIMENTAL PROCEDURE

Calibration of the pH meter: Follow the instructions provided by the manufacturer to calibrate your pH meter using a three-point method with buffers of pH=4, 7 and 10.

Solutions: You will be provided with two bottles of solutions for this assignment: a standardized NaOH solution and a smaller bottle of the unknown amino acid solution. Immediately, place your name and section on each label AND record the concentration of the standardized base and the number of the unknown amino acid solution in your notebook. You must keep both solutions until the official end of the assignment even if you finish early. Also, keep the solutions capped unless you are pouring the base solution into the burette or pipetting the amino acid solution into the titration beaker. At the end of the first lab period, put a rubber band around the two bottles before storing them on the shelf. Do not discard your bottled solutions.
Titration of the amino acid: The amino acid unknown solutions have been prepared in dilute hydrochloric acid solution. This lowers the pH, retards bacterial growth, and increases the stability of the unknowns. As a result, the amino acid in the solution will be partially in the protonated form (H₂A⁺) and partially in the neutral form. You should observe two endpoints (one very distinct and the other quite subtle) in your titration with base.

Rinse the burette with a SMALL amount of the base solution and discard the rinse in a waste beaker. Then fill the burette to between 0.00 - 1.00 mL. Read the precise level of the solution.

Pipet a 10.00-mL aliquot of your unknown amino acid into a 125-mL beaker. Add 20 mL of distilled water to ensure that the electrode tip is covered, and record the pH of the initial amino acid solution and the volume reading on the burette. Titrate the solution with the standardized NaOH solution taking pH and volume readings every 0.3 pH units. Rinse the sides of the beaker frequently during the titration to ensure that all of the analyte is in the solution. Titrate to a pH-value of 12. Stop the titration and refill the burette if you reach the 24.5-mL mark on the burette if the pH has not reached 11.5 units.

**Why does adding water to the solution not affect the determination of the concentration or pKₐ-values?**

Repeat the titration twice or until the average deviation of the volumes required reaching the first equivalence point is less than 0.10 mL.

Neutralize all titrant solutions with citric acid until the pH paper turns light green or orange before discarding the solution in the drain. Pour the small amount of waste NaOH used to rinse the burette into the labeled waste container. Do not pour un-neutralized NaOH solutions down the drain.

At the end of the assignment, place the capped bottles of unused NaOH and amino acid on the lab cart for return to the Lab Support facility.

**ASSIGNMENT PREPARATION AND DOCUMENTATION**

Refer to the Course Web Site for additional details regarding assignment due dates, specific homework study questions, modifications to the procedure, and tutorials and guidelines on the data and error analysis sections of the final report.

**Study Questions:**

On separate lined paper, complete any assignment-related study questions posted on the course website. Staple these to the copy of your notebook preparation work and turn them in at the start of the lab period.

**Lab Report (include these parts in the pre-lab for meeting 2):**

Preparation: Prior to your laboratory period, prepare your lab notebook for the experimental work involved in the determination of the concentration and pKₐ-values of an amino acid. Specifically, your notebook should include the following sections of the report:
**Introduction:** Include the goals of the assignment as well as the experimental techniques you will use to attain those goals.

**Procedure References:** Appropriately reference all primary and supplemental printed materials and any digital sites that you will use for the assignment.

**Flow Chart:** Prepare a flow chart of the assignment procedure including sufficient detail that you can use it as a checklist during the assignment to monitor your progress at each step along the way. In preparing your flowchart, place an asterisk beside each chemical for which you provide safety information in the next section of the report.

**MSDS Information:** In tabular format, provide the following information for each chemical that you will work with in the assignment:

- Product name
- Chemical formula
- Formula weight
- Melting point; boiling point and density
- Health hazard data (**summarize in your own words**)
  a) spill and disposal procedures (**summarize in your own words**)

**Experimental Records**

During the laboratory period(s) record the following information in your notebook and turn in a copy to your TA at the end of the laboratory period.

**Data and Observations:** In your notebook, record the unknown serial number of your amino acid. Record the concentration of the NaOH solution you were given.

For each titration, record in tabular form, the burette readings and readings of the pH meter observed during the titration.

**Analysis, Discussion, and Conclusions**

Following the completion of the experimental work complete the following components of the assignment report

**Graphs and Calculations**

*See the course website for Excel directions for plotting titration curves and first-derivative graphs.*

In order to determine the concentration of the amino acid, plot titration curves for your amino acid titrations. The difference in volume between the two inflection points is equal to the volume of base required to remove one proton from the amino acid. To precisely obtain these inflection points expand the vicinity of the equivalence points for your graphs so that you can read the scale on the x-axis to 0.02 mL—the precision of the burette readings.
If your pH measurements for the first equivalence point are precise, then the first derivative curve of the data should give you more reliable values for these equivalence point volumes of these titrations. Plot $\Delta \text{pH}/\Delta V$ as a function of volume for the equivalence point regions.

The pK$_a$-values of the amino acid are determined from the full titration graph.

To determine pK$_{a1}$ and pK$_{a2}$, locate the volume on the graphs half way between the two equivalence point volumes determined from the expanded derivative curves. The pH at this point is in the titration is equal to pK$_{a2}$. Now measure an equal distance on the graph to the left of $V_{ep1}$. The pH at this point is equal to pK$_{a1}$.

**Error Analysis:**

Calculate the relative average deviation in the concentrations of your amino acid.

Compare the relative average deviation with the inherent error calculated by propagating the errors in measurements of the pipet, the volumes determined from the graphs, and the standard base solution.

Estimate the absolute error in your pK$_a$-values by considering the variability you had in the pH’s of the solutions at the $|\Delta V_{ep}/2|$ points in the three titrations. Report the range for each of the pK$_a$-values.

**Conclusions**

Summarize your findings for the concentration of the amino acid and the pK$_a$-values and the errors associated with those values.