

## Understand how the charges of amino acids vary depending on pH

**Target Audience:** Recommended for undergraduate students of biology, biochemistry, or chemistry who are already familiar with the structure of amino acids.

**Estimated Time:** 10-20 minutes.

**Big Inquiry Question:** To what extent does the pH of the solution affect the net electric charge of an amino acid?

### Learner Activity

#### 1. Observe/Read

Amino acids contain amino ( $-NH_2$ ) and carboxyl groups ( $-COOH$ ) that can gain or lose protons ( $H^+$ ) depending on the pH of the solution. The isoelectric point (pI) is the exact pH value at which the amino acid has the same amount of positive charge as it does of negative charge, resulting in a net charge of zero. At this pH the amino acid exists as a Zwitterion.

#### 2. Predict

If an amino acid is added to a solution whose pH is significantly higher than its isoelectric point ( $pH > pI$ ), will its functional groups tend to gain or lose protons, and what total net charge will it acquire?

#### 3. Examine the Evidence

- Compare the protonation state of carboxyl and amino groups under acidic conditions versus basic conditions.
- Observe how the net charge shifts from positive to negative as the pH increases beyond the isoelectric point.

Consider a simple neutral amino acid like alanine ( $pK_{a1} \approx 2.35$  for the carboxyl group,  $pK_{a2} \approx 9.69$  for the amino group). At acidic pH, both groups are protonated with a net charge  $= +1$ . At neutral pH, the carboxyl group is deprotonated while the amino group remains protonated with net charge  $= 0$ . At basic pH, both groups are deprotonated with net charge  $= -1$ .

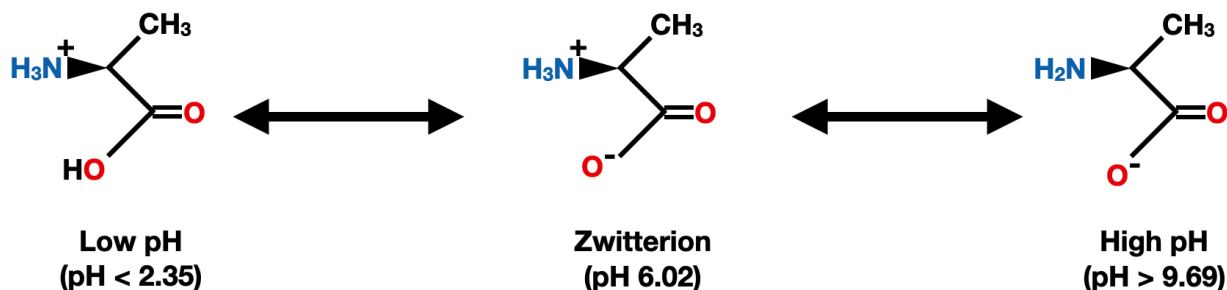


**Figure 1.** Effect of Solution pH on the Ionization State and Net Electrical Charge of Alanine.

#### 4. Explain

- How does the deprotonation of functional groups cause an increase in pH?

- Why does the loss of a proton cause the molecule's net electric charge to shift in the negative direction?

## 5. Reflect/Extend

How would the presence of an ionizable side chain (R-group), such as in aspartic acid or lysine, alter the titration curve and the determination of the net charge at physiological pH?

## Instructor/Teacher Guide

### 1. Purpose

To help undergraduate students understand how changes in environmental pH alter the protonation state of functional groups and determine the net electric charge of amino acids.

### 2. Materials

- Printed or digital copies of this activity guide.
- Basic scientific calculator or scratch paper for notes.

### 3. Facilitation Notes

- **Introduction (3 mins):** Briefly review what a zwitterion is and remind students of the definitions of protonation and deprotonation.
- **Independent or Pair Work (8–10 mins):** Allow students to work through the prediction, evidence examination, and explanation questions independently or in pairs. Encourage them to write out chemical structures if they get stuck.
- **Debrief (5 mins):** Lead a brief class discussion. Draw a generalized titration curve on the board and ask students to point out where the net charge is positive, zero, and negative. Highlight how Le Chatelier's principle is related to the amino acids charges.

### 4. Expected Responses

- **Prediction:** In a solution with a pH significantly higher than the pI, functional groups will tend to lose protons (deprotonate) because the concentration of  $H^+$  ions in the environment are low. The molecule will acquire a net negative charge.
- **Explanation:** -Deprotonation occurs when high pH (low  $[H^+]$ ) shifts the chemical equilibrium toward releasing protons into the solution.  
-Losing a positively charged proton ( $H^+$ ) removes positive charge from the species, making the overall net electrical charge more negative.
- **Reflect / Extend:** An ionizable R-group introduces a third  $pK_a$  value, creating an additional buffering region and shifting the overall isoelectric point. For instance, acidic amino acids will have lower pI values, while basic amino acids will have higher pI values, directly influencing their net charge at physiological pH.

### 5. Accessibility Notes

- Provide print versions of the titration data and/or chemical structures.
- Allow students to give their explanations written and oral.

- Ensure all chemical structures are described verbally during facilitation to support students with diverse learning needs.

#### **SDG 4 Connection**

This activity directly contributes to SDG 4: Quality Education, by promoting critical scientific literacy and active reasoning rather than rote memorization. By engaging college students in research-based problem-solving, it develops fundamental biochemistry skills and provides a reusable, open-access teaching tool that instructors can easily adapt to various educational settings.

#### **References**

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<https://biochemtools.com/amino-acid-chart.html>

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