pH and Protein Solubility

A Reversible Demonstration

Introduction

Any change in the pH of a protein's environment will cause observable changes in the solubility of the protein. These changes reflect changes in the three-dimensional structure of the protein. The effect of pH on protein solubility explains why most enzymes function well at an optimum pH, and why their activity decreases substantially at pH values other than the optimum.

Concepts

- Protein
- Solubility
- pH

Materials

Casein, 2 g

Hydrochloric acid, HCl, 3 *M*, 50 mL

Sodium hydroxide, NaOH, 0.01 *M*, 250 mL

Sodium hydroxide, NaOH, 3 *M*, 50 mL

Beaker, 600-mL

Magnetic stirrer and stirring bar

Pipets, Beral-type, 2

Universal indicator (optional), 2 mL

Safety Precautions

Hydrochloric acid is toxic by ingestion or inhalation and is severely corrosive to skin and eyes. Sodium hydorxide solution is a corrosive liquid and is very dangerous to eyes; causes skin burns. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

1. Place a beaker containing 250 mL of 0.01 *M* sodium hydroxide and a stirring bar on a magnetic stirrer. Stir at moderate speed. Add 2 g of casein and stir to dissolve. The solution will be slightly cloudy or translucent.
2. *Optional:* Add 1–2 mL of universal indicator to monitor pH, if desired. Consult the universal indicator color chart to follow the solubility of casein as a function of pH.
3. Add 3 *M* hydrochloric acid one pipet-full at a time using a Beral-type pipet. A white precipitate will form after the addition of about 10–15 mL of hydrochloric acid. The pH at which the precipitate appears is 4–5.
4. Continue adding hydrochloric acid with stirring. The precipitate will redissolve after the addition of another 15–20 mL of acid (pH <2).
5. Reverse the process by adding 3 *M* sodium hydroxide with stirring. The protein will precipitate out again after about 20–30 mL of sodium hydroxide have been added. This occurs as the pH increases through the 4–10 range.
6. Continue adding sodium hydroxide dropwise with stirring. The precipitate will redissolve as more base is added and the pH increases above pH 10–12.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. The casein solution may be stored at basic pH for several months. Alternatively, the solution may be rinsed down the drain with excess water according to Flinn Suggested Disposal Method #26b.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12

Evidence, models, and explanation

Publication No. 10482
061616

Content Standards: Grades 5–8

Content Standard A: Science as Inquiry
Content Standard B: Physical Science, properties and changes of properties in matter
Content Standard C: Life Science, structure and function in living systems
Content Standard F: Science in Personal and Social Perspectives

Content Standards: Grades 9–12

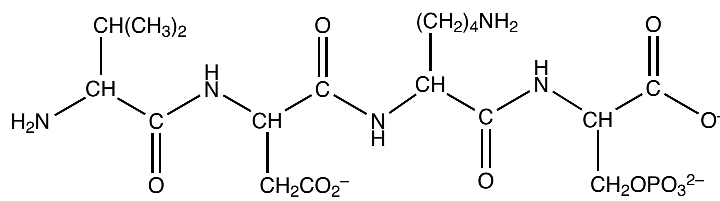
Content Standard A: Science as Inquiry
Content Standard B: Physical Science, structure and properties of matter
Content Standard C: Life Science; matter, energy, and organization in living systems, behavior of organisms
Content Standard F: Science in Personal and Social Perspectives

Discussion

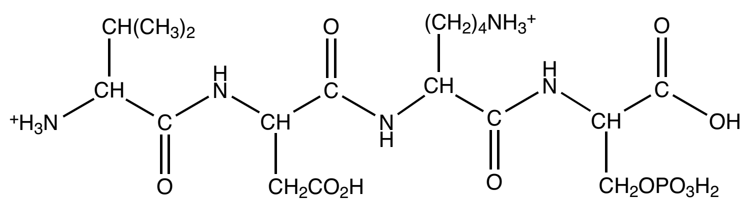
Casein is the principal protein in milk, comprising 80% of the total protein content in milk. Casein is a phosphoprotein—it contains a large number of phosphate groups attached to the amino acid side chains in its polypeptide structure. The negatively charged phosphate groups are balanced by positive calcium ions and are responsible for the high nutritional calcium content in milk. Casein is almost completely insoluble in water at neutral pH (pH = 7).

Casein, like other proteins, is an ionic species containing amino groups and carboxyl groups on its terminal amino acids. It also contains a variety of other acidic and basic groups on the side chains of its non-terminal amino acids. The effect of pH on the solubility of casein reflects the ionization of the acidic and basic groups in its structure.

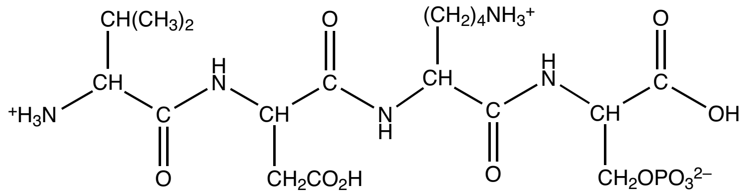
At high pH, casein will have a net negative charge due to ionization of its acidic side chains (—CO_2^-). Because casein is ionized at high pH values, it is soluble in dilute sodium hydroxide solution.



At low pH, casein will have a net positive charge due to protonation of its basic side chains (—NH_3^+). Because casein is ionized at low pH values, casein is also soluble in strongly acidic solutions.



At intermediate pH values, casein will contain an equal number of positively and negatively charged groups and the protein will have a net charge of zero. Casein is insoluble in neutral solutions because it is not charged under these conditions.



The solubility of a protein is usually at a minimum at its isoelectric point. The isoelectric point is defined as the pH at which a protein has a net charge of zero. For casein, due to the attached phosphate groups, the isoelectric point is close to pH = 4.

Materials for *pH and Protein Solubility—A Reversible Demonstration* are available from Flinn Scientific, Inc.

Catalog No.	Description
C0043	Casein, 100 g
H0034	Hydrochloric Acid, 3 M, 500 mL
S0447	Sodium Hydroxide, 3 M, 500 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.